United States
Department of
Agriculture

Forest Service

Forest Pest
Management

WORK PLAN

Bacillus thuringiensis - Drift/ Dispersion and Effects on Non-Target Lepidoptera, Utah 1993 Phase III

Healthy Forests
Make A World
Of Difference

FPM 93-8
April 1993

Pesticides used improperly can be injurious to human beings, animals, and plants. Follow the directions and heed all precautions on labels. Store pesticides in original containers under lock and key—out of the reach of children and animals—and away from food and feed.

Apply pesticides so that they do not endanger humans, livestock, crops, beneficial insects, fish, and wildlife. Do not apply pesticides where there is danger of drift when honey bees or other pollinating insects are visiting plants, or in ways that may contaminate water or leave illegal residues.

Avoid prolonged inhalation of pesticide sprays or dusts; wear protective clothing and equipment, if specified on the label.

If your hands become contaminated with a pesticide, do not eat or drink until you have washed. In case a pesticide is swallowed or gets in the eyes, follow the first aid treatment given on the label, and get prompt medical attention. If a pesticide is spilled on your skin or clothing, remove clothing immediately and wash skin thoroughly.

NOTE: Some States have restrictions on the use of certain pesticides. Check your State and local regulations. Also, because registrations of pesticides are under constant review by the U.S Environmental Protection Agency, consult your local forest pathologist, county agriculture agent, or State extension specialist to be sure the intended use is still registered.

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Bacillus thuringiensis - Drift/ Dispersion and Effects on Non-Target Lepidoptera, Utah 1993 Phase III

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PREFACE

This plan covers field procedures to study the drift/dispersion of Bacillus thuringiensis (Bt) in mountain terrain during June 1993. The plan also includes procedures to study the effects of Bt on non-target Lepidoptera. The project will be conducted in Mill Creek Canyon, Salt Lake County, in conjunction with the 1993 Utah gypsy moth eradication project. The biological pesticide Bt Dipel 6AF will be applied by helicopter to 856 acres in a treatment block designated SL-2. Bt will be sprayed under operational conditions of the eradication project. The drift/dispersion study is in follow-up to recommendations from the National Spray Model Steering Committee, National Steering Committee for Managing Western Defoliators, and National Steering Committee for Gypsy Moth and Eastern Defoliators. Scientists from the USDA-FS and U.S. Army, in cooperation with Utah State Department of Agriculture and contractors will participate in this study. No special spray treatment will be applied and no tracers will be added to the spray tank mix for the benefit of these studies. Spray drift/dispersion will be monitored down-canyon to quantitate spray movements. This is a continuation of studies that began in Utah in 1991. This study plan may be modified as needed and as agreed to by the cooperators. Results will be published in the open literature and presented at professional meetings.

INTRODUCTION

Off-target movement of pesticides from forest spray operations has been a concern since aircraft were first used to spray trees (Neillie and Houser 1922). The concern primarily centers on potential environmental impact of pesticides on non-target species. Biological pesticides, such as Bacillus thuringiensis (Bt), are not exempt from this concern. Assessing potential environment impact of pesticides first requires quantitative data on the amount of pesticide that moves off the target site, followed by conducting environmental impact evaluations. Off-target movement is also a concern as it represents an inefficient use and economic waste of pesticides. For these reasons data are also needed to quantitate off-target movement that may lead to improving the safety, efficiency and efficacy of aerial spray operations; and to establishing spray buffer zones. Predictions of the Forest Service Cramer-Barry-Grim (FSCBG) aerial spray model Teske (1990) and Teske et al. (1993), a computer model that predicts travel and deposition of aerial sprays, have been compared favorably to several sets of observed field data and reported by GCA Corporation (1971); Boyle et al. (1975); Dumbauld et al. (1976); Dumbauld et al. (1977); Rafferty et al. (1987), Rafferty et al. (1988), Rafferty et al. (1989), and Teske et al., (1991), and Rafferty and Bowers (1993).

Sampling off-target drift of pesticides in forests and in mountain terrain presents technical challenges. Researchers have had relatively few opportunities to obtain such data in mountains and relatively few references are available in the literature, particularly those involving biological aerosol studies (Sassaman, 1987). In situations where researchers have tried, results have been somewhat disappointing, due to a variety of reasons, including type of tracers and samplers used, sample contamination, and inadequate weather monitoring. Spray drift resulting from treatment of coniferous seed orchards has been reported by Barry et al. (1983); however the reported tests were conducted in relatively flat terrain. Rafferty et al. (1988) reported deposition drift downwind to 2500 meters. A study similar, as to the one outlined in this study plan, was conducted in an adjacent canyon in 1991 (Barry 1991). Results of this study (Barry 1993) clearly demonstrated that detectable amounts of Bt drifted at least 3,150 meters downwind from the treatment block. This is a continuation of the 1991 study and with additional tasks.

OBJECTIVE/TASKS

The objective is subdivided into seven tasks as listed below:

Task 1 - to evaluate the effects of Bacillus thuringiensis (Bt) on selected non-target Lepidoptera species in the Wasatch Mountain Range of northern Utah.

Task 2 - to quantify the deposition of Bt on host foliage of non-target Lepidoptera and to compare results with FSCBG model predictions of deposition on host foliage.

Task 3 - to evaluate size of buffer zones needed to protect non-target Lepidoptera colonies from Bt spray drift.

Task 4 - to compare FSCBG model drift predictions to dosage recoveries from Rotorod spinning samplers; and deposition recoveries from Mylar deposit samplers and Gambel oak foliage.

Task 5 - to evaluate the ValMet module of FSCBG dispersion predictions by comparing predictions to Bt deposition and airborne dosage.

Task 6 - to evaluate relationship of Bt recoveries among sampler types and Gambel oak foliage.

Task 7 - to investigate residual Bt aerosols that might persist at mouth of Mill Creek Canyon after treatment.

SCOPE

The study and treatment sites are located in Salt Lake County, Utah, R.1E., R.2E., T.1S., Sections 28, 33, 29, 32, 31, and 36. These sections, composed of public and private lands, are located in Mill Creek Canyon and down-canyon to a distance of 4900 meters to its intersect with Wasatch Blvd. Terrain of the study area is mountainous ranging in elevation from 4,900 to 5,800 feet MSL. Mill Creek Canyon consists of mixed conifers, Gambel oak, big tooth maple, and a variety of other woody species. The site is ideally suited for these studies due to topography, channeling of drainage winds, and physical access.

The treatment site, designated as the Mill Creek Spray Block (SL-2), consists of 856 acres. After the first spray is applied to eradicate gypsy moth, treatment will be repeated two additional times at approximately five day intervals thus providing an opportunity for three replication trials. The drift/dispersion and non-target Lepidoptera studies will be conducted down-canyon from the SL-2 treatment block. Spray moving from the treatment area will be sampled by a variety of samplers positioned downwind to approximately 5350 meters. Exact location of the selected sampling stations will be determined by GPS technology. Weather will be monitored during the study with a variety of instruments.

US Army Dugway Proving Ground (DPG) will be contracted to provide field crews sampling equipment, laboratory support, and meteorological instruments. MTDC and RM Station will also provide meteorological instruments. A dry run will be conducted before May 30, 1993 to brief field crews, to practice field procedures, and to coordinate activities.

METHODS - DEPOSITION/DOSAGE SAMPLING

Application

The SL-2 Mill Creek Block will be treated operationally with Bt pesticide, applied undiluted by helicopter at 0.5 gallons per acre. The Bell 206 B III helicopter will be calibrated to apply at swath widths or lane separations of 30.5 meters. The helicopter will be equipped with 4 each Beecomist 365A rotary atomizers and will fly a 70 mph ground speed. A total of 1284 gallons will be applied to SL-2 during the three treatments. Success of the study is dependent upon an organized drainage wind that results from nighttime cooling of the ridges and canyon slopes. To increase potential for a successful study the Treatment Supervisor's is requested to:

1. Begin each of the 3 applications of SL-1 at first light when the pilots believe it is safe to fly and complete spraying the entire block before upslope winds begin.

2. Avoid spraying SL-2 if cloud cover precludes surface cooling. Drainage wind depends upon surface cooling that might not occur if clouds hold warm air near the surface.

3. Complete spraying in SL-2 before up-canyon winds develop.

Spray Material (Tank Mix)

The Bt to be applied Dipel 6AF (see attached pesticide label in Appendix) is a commercial water-base formulation, with a potency of 12,000 infectious units per milligram equivalent to 48 billion international units (BIU) per gallon. It will be applied undiluted at the rate of 0.5 gallons (24 BIU) per acre. No tracers or other additives will be added to the tank mix. The material has a relatively low rate of volatility and high specific gravity. Wind tunnel testing of the atomization under conditions (atomizer, air speed, and application rate) approximating the anticipated operational conditions, is provided in Appendix.

Meteorology Support

This section describes the methods to collect meteorological data which will be used to study the physical phenomenon of pesticide spray drift. Evaluation of the FSCBG spray drift model and ValMet module will also be emphasized. Specific attention will be given to validation of the ValMet module which will simulate atmospheric transport in complex terrain within the framework of the main FSCBG model. In the past, FSCBG validation experiments have been conducted with meteorological data collection consisting of a number of point measurements of mean wind speed, direction, relative humidity, air temperature; and occasionally solar and/or net radiation measurements and some upper air data (most recently using a tethersonde). This was not adequate as it is difficult to track the spray without having a detailed understanding of the atmosphere structure within the lower atmosphere and mixing layer. In complex terrain, the vertical structure of the atmosphere is considerably more complex than over flat terrain. FSCBG allows for input of mixing depth; therefore, upper air measurements should be conducted as part of any validation study. The in-canopy and near canopy structure of the atmosphere is also complex. FSCBG accepts mean wind data from release height down through the canopy profile data, to account for the effect of the large variation in meteorological parameters near the canopy on spray drift. These profile measurements will be made as part of a spray drift validation study.

Upper Air Data

Free convective eddies in the daytime surface layer can provide the upward momentum to cause neutrally or near-neutrally buoyant materials to rise and become entrained in the flow above the canopy in the zone where aerial spray is released. For these reasons, it is important to understand the nature of the atmosphere above the target canopy. Material which is lost 'out of the top' is available for long range drift.

In the western U.S., most aerial spray projects managed by the Forest Service are conducted in complex terrain where the upper air patterns are dependent on fetch, slope aspect and synoptic conditions. Small changes in wind direction will alter the 'memory' of the flow (change the projected shape of obstacles, thus altering the wake, frontal and lateral vortex field). Local changes in insolation (due to cloud cover, etc.) will influence the strength of valley flow cells. Thus, the upper air movement is difficult to anticipate or even interpolate over large distances. The Forest Service has invested in the development of the ValMet model through cooperation with the Department of Energy to describe atmospheric transport in complex terrain. This is a phenomenological model that simulates valley flow through the description of flow tubes which are oriented along the valley axis. Suitable field data are needed to evaluate this model.

Canopy Meteorology

There are basically four distinct layers in and immediately above most forest canopies. The lowest is the trunk space which typically has relatively low stem area and may be intermittently coupled/decoupled with the driving flow above the canopy depending on canopy density and proximity to canopy holes or edges. The next higher layer is a shear layer at the base of the densest foliage. When a pulse of momentum moves through under the canopy, the momentum flux may actually be reversed in this layer (counter-gradient momentum flux). The third layer up is the layer of densest foliage and is often the layer with the lowest mean velocity in dense canopies. The fourth layer is a strong shear layer where the vertical velocity gradient is very steep and velocity increases rapidly with height near the top of the canopy. Above this layer the air flow adjusts to the free atmosphere.

By considering the mean velocity profile, the transport distance per unit time may be implied for the different layers. However, to calculate dispersion (dilution) and likelihood of spray drop impaction on a surface, turbulence should be considered. The concentration of a scalar contaminant will be dependent upon the turbulent regime in which it is transported. In the layer of dense foliage, mean velocities are low and vortices tend to be small due to the size of the foliage elements. Above the canopy, shear eddies blend into larger boundary layer scales. It should also be noted that the larger boundary layer motions will occasionally penetrate all canopy layers and mix in air from above.

Due to the differences in the relevant physical parameters between canopy layers, the description of pesticide transport and deposition in and near the canopy is greatly enhanced if a detailed, vertical meteorological profile is available.

Sub-Tasks Meteorology Support

The sub-tasks of this meteorology support are:

1) To provide inputs to FSCBG that allow modelers to minimize the use of defaults in operating the model.

2) To obtain a data set that will be useful in validating the ValMet complex terrain module and other model improvements that are currently being developed.

3) To obtain a data set that will be useful in indicating future developmental directions to improve spray operations.

4) To achieve effective technology transfer through the timely dissemination of project reports and model enhancements.

5) To present one paper at an appropriate technical conference and to prepare an article for publication in an appropriate scientific journal.

Methods and Instrumentation

The proposed site is Mill Creek Canyon, just east of Salt Lake City, Utah; a narrow canyon with a forested floor and steep sides, talus in places, and largely grass and scrub covered or bare rock. The canyon mouth opens directly onto the upper bench of the Salt Lake Valley.

This paragraph describes the instrumentation necessary to achieve the stated sub-tasks. A basic instrument station (EMCOT, Ekblad el at 1990) has been operated on FS spray projects by MTDC for a number of years. This station consists of a cup anemometer, a vertical propeller, two thermistors to measure two levels of air temperature and a humidity sensor. MTDC proposes for this study to upgrade this station by substituting a higher resolution cup anemometer and adding net radiation to the station (net radiation is an input to FSCBG but a default calculation is often substituted).

Profiling of canopy turbulence will be accomplished with three-dimensional sonic anemometers provided by DPG. Four anemometers will be used to measure wind and turbulence within and above the canopy. These instruments measure the change in an acoustic wave due to fluid velocity across a 15 cm pathlength. By using a three axis sensor, the total vector is determined. DPG will also provide a scintillometer as part of the instrumentation plan. This instrument uses a light beam to measure flow perpendicular to the beam. This instrument should be very useful in this situation since the valley flows often follow the valley axis and a beam oriented across valley would be perpendicular to this along valley flow. In conjunction with the turbulence measurements, high frequency temperature measurements and one level of fluctuating pressure will be measured.

The upper-air motion in the valley will be obtained using acoustic sounders. These instruments use the reflection of sound waves to determine the upper-air motion. Depending on the type of sounder used and the range settings, these instruments will measure motion from a low level between 10 and 50m and an upper level on the order of hundreds of meters. Two of these instruments will be deployed. At least one will be a PA1 (Phased Array n°1) phased sounder, the other will be selected at a later date.

The positioning of the instruments (Table 1) will be determined largely by site geography and logistics. A site walkover will occur during March 1993 involving DPG, Region 4 and MTDC. Specific location of meteorological instrumentation will be determined during April after site visits. Locations will be fixed by GPS. One will probably be stationed on a hillside above the vegetation. Security is a major concern, especially for the more sophisticated equipment.

Table 1. Summary of instrumentation selected for the 1993 R-4, gypsy moth project.

Instrument	Sampling Frequency (Hz)	Number Deployed	Purpose
Cup Anemometers 12 Slot Photochoppers	2	3	Wind Speed input to FSCBG and ValMet
Cup Anemometers Magnetic	1	3	Wind Speed input to FSCBG and ValMet
Propeller Anemometers (single propeller)	1	3	Wind Elevation angle input to FSCBG
Wind Vanes	1	6	Wind Direction input to FSCBG and ValMet
Sonic Anemometers (10 or 15cm pathlength)	20	4	Turbulence input to FSCBG and ValMet (Speed and Direction)
Temperature (Assorted)	>1	>6	Temperature inputs to FSCBG and ValMet
Humidity	1	3	Humidity input to FSCBG
Net Radiometers	1	3	Net Radiation input to FSCBG

Meteorological Instrumentation Summary (cont.)

Scintillometer	.01	1	Above canopy, valley core Wind Speed and Temperature input to ValMet
Sodar Phased Array n°1 (PA1)	.001	2	Upper air structure. Mixing Height input to FSCBG and comparison to ValMet
Pressure	2	1	Pressure input to FSCBG and ValMet
Raingauge	.0001	1	Adjunct to insect exposure stations

Instrument Arrays

MTDC will deploy three EMCOT towers. These towers are each 7-m high and each have two levels of wind speed, wind direction and temperature. Included on these towers are net radiation and humidity. These instruments are recorded using a Campbell Scientific CR-10 data logger. They have the option of radio telemetry for real time data monitoring or data can be stored for later retrieval.

DPG will supply a 17-m tower. On this tower, four levels of wind speed and wind direction will be measured using sonic anemometers. Also included on this tower will be four levels of temperature, measured by using oscillating quartz thermometers. These instruments will provide turbulence and flux information through the forest canopy. Associated with these instruments will be a high frequency pressure transducer.

Instrument Location

17-m Dugway tower Located in the canopy on the valley bottom at the down valley edge of the spray block. This is the eastern end of the main part of the Tracy wigwam boy scout complex.

EMCOT Station 1 Located north of the canyon road on a north-south trending spur overlooking the main boy scout complex.

EMCOT Station 2 Located in a gas company enclosure between Wasatch Blvd. and Hghwy. 215 west of the canyon mouth.

EMCOT Station 3 This station will be used to increase coverage outside the canyon or positioned on a north facing canyon wall midway between the spray block and the canyon mouth.

Ft. Collins SoDAR Located at the west end of the pond in the main boy scout complex on the valley floor.

RemTech phased array SoDAR Located on private property, north of the road immediately west of the canyon mouth.

Scintillometer Positioned on the canyon sides to measure across valley above the Ft. Collins SoDAR. (Across the west end of the main boy scout complex.)

Some adjustments may be made in positioning instrumentation.

Sampling

Approximate location of the sampling stations is shown in Figure 1. Off-site movement of Bt will be sampled downwind to 5350 meters (3.32 miles) for dosage with spinning "U"-shaped brass Rotorod samplers; and for deposition with Kromekote cards and Mylar sheets (Figure 2). Twenty-two sampling stations consisting of 11 pairs, will be positioned at 11 locations along a line that begins on the downwind edge of the SL-2 block and follows the drainage down-canyon intersecting at Wasatch Blvd. A branch of Gambel oak leaves will be attached to a tether between the Rotorod sampler stakes (Figure 2).

Samplers will be positioned and set-out the morning of treatment and picked-up after the spray operations and spray cloud passage is complete. The Test Officer will follow the sampling schedule (Table 2). The schedule assumes a 5 meter per second mean transport wind in activating and deactivating the samplers to insure sampling the entire spray cloud.

Sampling station (Figure 2) will consist of the following:

1. 2 each "U"-shaped brass Rotorods spinning clockwise at 2400 rpm, potentially sampling 120 liters per minute.

2. 2 each Kromekote cards on cardboard.

3. 2 each Mylar sheets on cardboard.

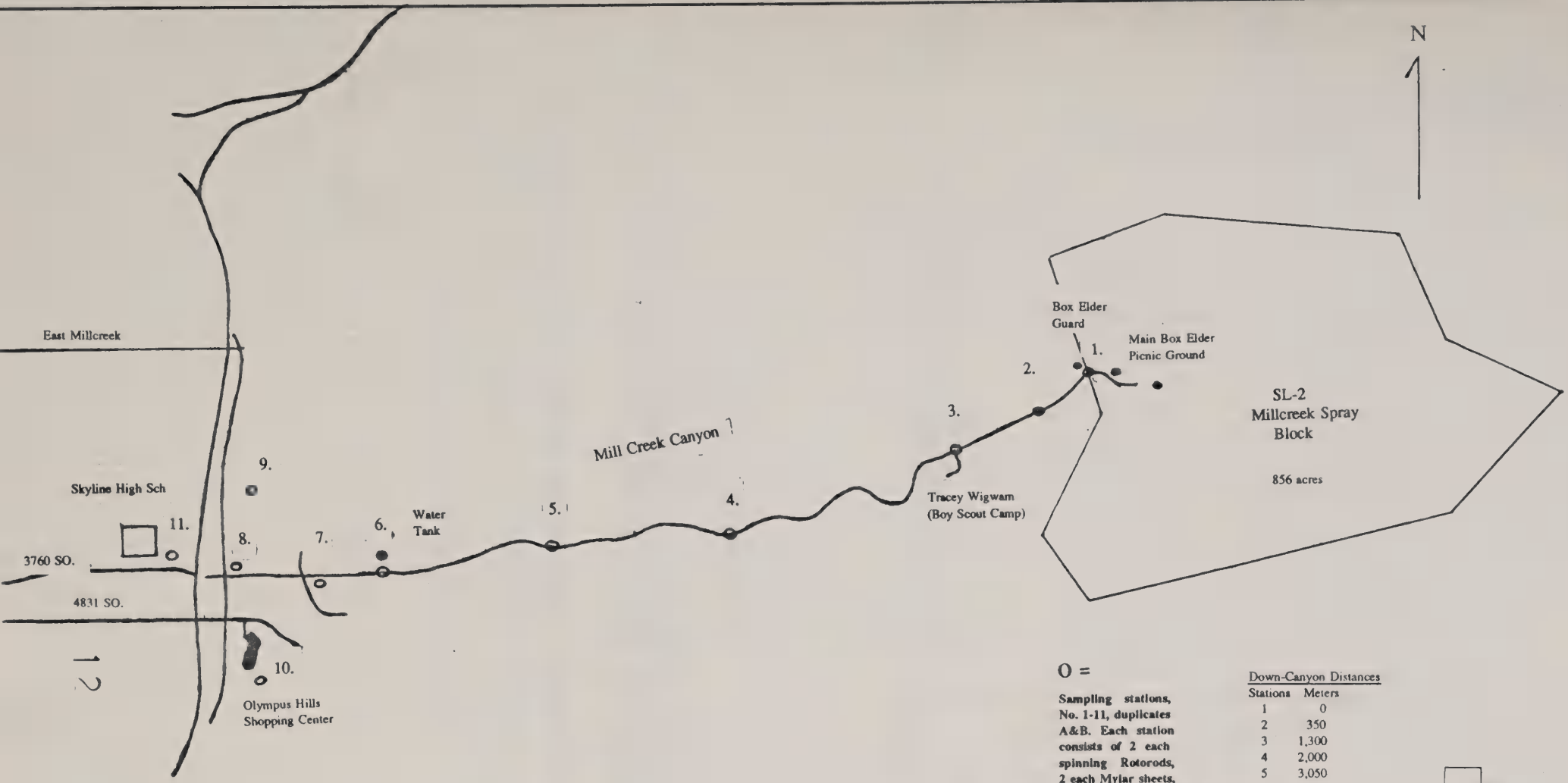
4. 2 branches approximately 8" in diameter of Gambel oak each containing several leaves collected morning of treatment. Five extra branches will be collected each morning as controls. These will be kept in paper bags, sealed, and protected from the spray and other possible sources of contamination.

The Rotorods will be elevated at 1.5 meters above the ground. The Kromekote cards and Mylar sheets will be attached to cardboard holders and in-turn these will be placed on boards at ground level (Figure 2). The board will insulate the samplers from moisture and help to reduce shielding from plants.

Rotorod Sampler

The "U"-shaped brass Rotorod sampler, developed by Metronics, and currently produced by Ted Brown Associates, is a rotating arm impaction device capable of obtaining quantitative data of airborne particulates in the size range $\geq$ 10 microns. At a nominal 2400 rpm which moves the collecting surfaces through the air and thus causes particles within the air intercepted by the collector rods to become impacted on the leading flat-surfaced edges of the rods. It samples 120 liters per minute when rotating at 2400 rpm. The collecting surface of the "U"-shaped rod is 0.159 cm wide. Its basic components are a constant speed motor and aerodynamically designed collector rods which are rotated by a 12-volt motor (Appendices 3 and 4). Edmonds, (1972) describe the sampler and provide instructions for its installation, operation and evaluation.

Figure 1 - Site map 1993 Utah Spray drift and non-target study.



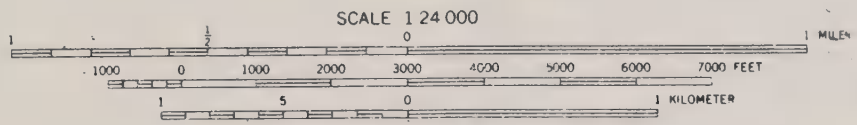
O = Sampling stations, No. 1-11, duplicates A&B. Each station consists of 2 each spinning Rotorods, 2 each Mylar sheets, 2 each Kromekote cards, and host foliage for the insect feeding stations. 1A&1B located at Box Elder Guard Station.

Down-Canyon Distances

Stations	Meters
1	0
2	350
3	1,300
4	2,000
5	3,050
6	4,100
7	4,450
8	4,900
9	4,900
10	4,900
11	5,350

QUADRANGLE LOCATION

TM GRID AND 1975 MAGNETIC NORTH DECLINATION AT CENTER OF SHEET



CONTOUR INTERVAL 40 FEET
NATIONAL GEODETIC VERTICAL DATUM OF 1929

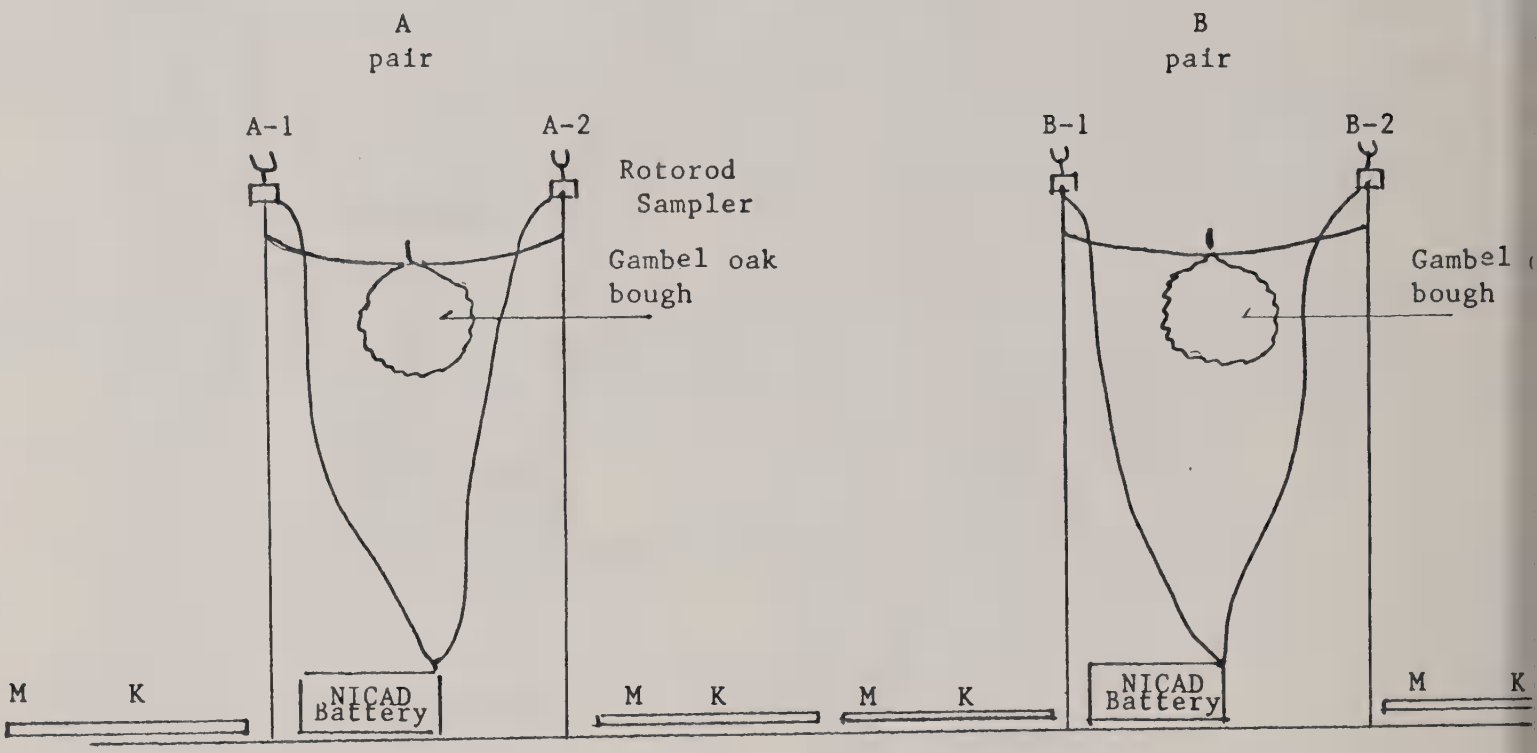
SITE MAP 1993 UTAH

SPRAY DRIFT & NON-TARGET STUDY

U. S. DEPARTMENT OF AGRICULTURE

Designed
Drawn J.Barry
Traced
Checked
Date 3-17-93
Approved by
Title
Sheet
Drawing No
No.
of

FS-6130-A REV 8-75



Notes:

- There will be 11 of these set-ups as shown.
- Samplers (Rotorods, Mylar, and Kromekote cards) should be positioned perpendicular to axis of the drainage winds to avoid one sampler apparatus shielding the next. R=Rotorod, M=Mylar, and K=Kromekote.
- Nicad battery will be used to power the Rotorod motors.
- Gambel oak leaves will not be used for insect feeding study.

Figure 2 - Diagram of a duplicate sampling station, 1993.

Table 2 - Sampler activation/deactivation times in minutes based upon a wind speed of 5 meter per second assumed mean transport wind.

Station	Distance[1] Downwind (m)	Cloud[2] Arrival (min)	Sampler Activation (min)	Cloud[3] Passage (min)	Sampler[4] Deactivation (min)
1 A&B	0	Z	Z-5		
2 A&B	350	1.7	Z-5		
3 A&B	1350	4.5	Z-5		
4 A&B	2000	6.7	Z-5		
5 A&B	3050	10.2	Z		
6 A&B	4100	13.7	Z		
7 A&B	4450	14.8	Z		
8 A&B	4900	16.3	Z+5		
9 A&B	4900	16.3	Z+5		
10 A&B	4900	16.3	Z+5		
11 A&B	5350	17.8	Z+5		

Notes:

1. Distance downwind from west and down-canyon edge of SL-2 Mill Creek Canyon.
2. Z = Begin of spraying, all times relative to Z in minutes.
3. Cloud passage = cloud arrival + number of minutes required to complete morning spraying.
4. Sampler deactivation = cloud passage + 20 minutes.

The Rotorod samplers will be used at each sampling station to sample airborne Bt particles through impaction. It will be uncoated. All Rotorods will be elevated 1.5 meters above ground.

The Rotorods will be used in pairs at each duplicate station. The rotating Rotorods will be connected to a 12-volt motor and powered by a 12-volt NICAD battery.

Mylar Sheet

Mylar sheet, measuring 4 5/16 x 6 9/16 inches provided by the USDA-FS, will be positioned at ground level in duplicate (Figure 2). The Mylar will collect deposition of Bt spores resulting from gravitational settling.

Kromekote Cards

Kromekote Cards, measuring 4 5/16 x 6 9/16 inches, will be placed at each sampling station to collect particles that might deposit by gravitational settling. The cards will be placed in plastic holders and positioned horizontally at ground level in duplicate, on a flat piece of plywood. Kromekote cards and holders will be provided by the USDA Forest Service.

Oak Leaves

A Gambel oak branch approximately 8" in diameter and length will be attached between each Rotorod stake immediately below the Rotorod (Figure 2). The branches will be collected from a non-treated area.

Quality Control

Quality control includes both handling and exposure of control samplers and samples to detect Bt background, natural and accidental contamination of samples by Bt. Field control samples will be taken and standard DPG Life

Sciences Laboratory operating procedures will be followed to monitor contamination and quality control in handling samplers and in conducting assay. A spinning Rotorod control will be operated for one hour prior to spraying at every even numbered sampling station to monitor Bt background and contamination. Controls will be packaged, marked and removed from the sampling area prior to spraying. A Mylar sheet control sampler also will be placed at every even numbered sampling station while setting up the station and picked up prior to commencement of spraying. A laboratory data report should also include results of laboratory control samples.

Review of the 1992 laboratory assay data suggest need for improved quality control and prevention of sampler contamination in the field. Under supervision of the DPG Project Officer and Crew Leader personnel should be especially aware of:

1. **Need** to pick-up all equipment needed for the trial using equipment inventory record sheets.

2. **Need** to locate samplers to avoid contamination caused by secondary aerosols.

3. **Need** to report all discrepancies e.g. contaminated and missing samples, and sampler failures.

4. **Need** to follow the sampler marking system per this Study Plan; and

5. **Need** to report sampler on and off times.

6. **Need** to follow sterile procedures in handling samples.

7. **Need** to keep crews and vehicles upwind or out of the canyon during spraying and sampling.

8. **Need** to inventory samples after each trial.

9. **Need** to realize that Mill Creek Canyon will be contaminated after the first treatment.

A liter of tank mix that represents the batch of Bt applied each day to SL-2 will be collected from the spray aircraft by the Utah State Liaison Officer and sent to the DPG laboratory for analyses. The sample should be placed in a plastic bottle, double packaged in plastic bags, and transported in an ice chest. The sample should be dated and Bt lot number placed on label.

Avoiding Contamination

The most serious threat to the integrity of the sampling is contamination of samples with Bt. Bt is a spore former that commonly occurs in the soil, thus there is potential for contamination even from naturally occurring Bt. But the biggest threat is from Bt contamination after the first treatment. Being a spore former Bt is persistent as opposed to vegetative cells that are more susceptible to UV radiation and other degrading factors. Potential of contamination before the first spraying is minimal as Bt is not used at the DPG laboratory. But once Bt is released contamination is a serious threat. After treatment personnel and the entire study area including foliage , soil, vehicles, clothing, etc. and other surfaces will be contaminated. Potential sources of sampler contamination include contaminated equipment, (sampling equipment, vehicles, containers) non-sterile samples, secondary aerosols (natural and man made), improper handling, packing, transportation of samplers, and contaminated crews (skin and clothing). Suggested procedures to reduce potential for contamination include:

1. Follow Laboratory Officers instructions on handling and transporting samplers.

2. Clean and sterilize Rotorod samplers. If Rotorods are accidentally contaminated by dropping on ground, touching with contaminated hands or gloves, etc. this should be reported to the Test Officer and marked on the sampler bag. Always use sterile gloves or ziploc bags between Rotorods and your hands. Carry extra sterile Rotorods.

3. Avoid creating dust and secondary aerosols near samplers.

4. Approach samplers on downwind side.

5. Insure that Mylar card holders are sterile after each treatment.

6. Handle Mylar with sterile instruments (e.g. forceps) or sterile surgical gloves, remembering that once the exposed Mylar is touched the instrument or glove is contaminated.

7. Wear clean clothing daily.

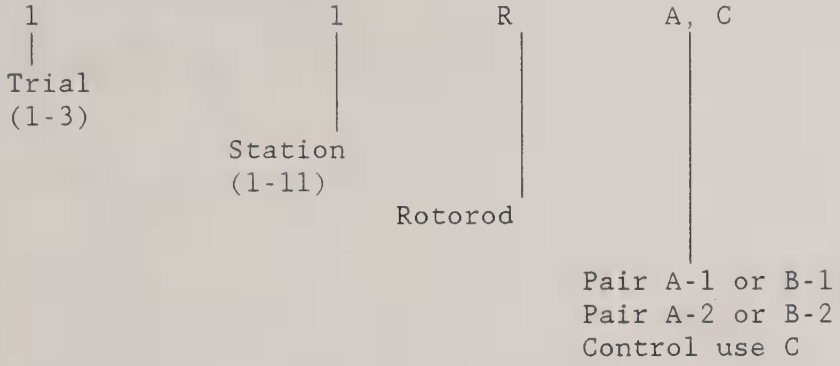
8. Keep vehicles and personnel upwind or out of the canyon during trials - avoid the spray cloud.

9. Wash-down vehicles, racks, tote boxes, and other equipment after each spray day. This will help to reduce potential of contamination from secondary aerosols and cross contamination.

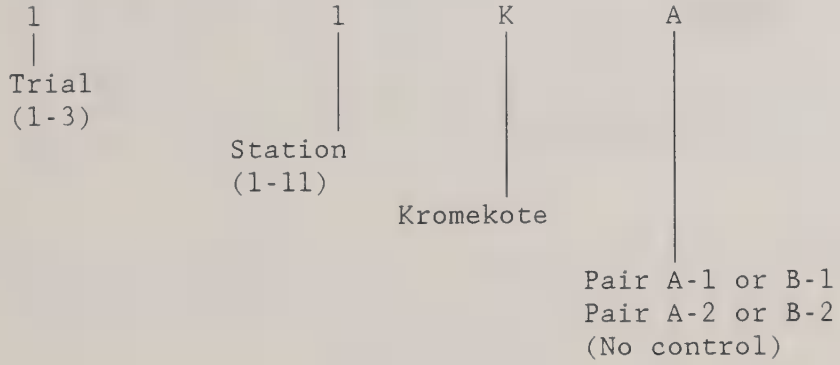
Sampler Marking

Sampler marking codes will be used throughout the study from the laboratory where the sampler is prepared through to the reporting of data. Codes will be marked on the outside of the Ziploc bags that contain Rotorod and Mylar samplers, and paper sacks that contain leaf samples. Errors in following the marking procedures will result in lost data. (See Figure 2)

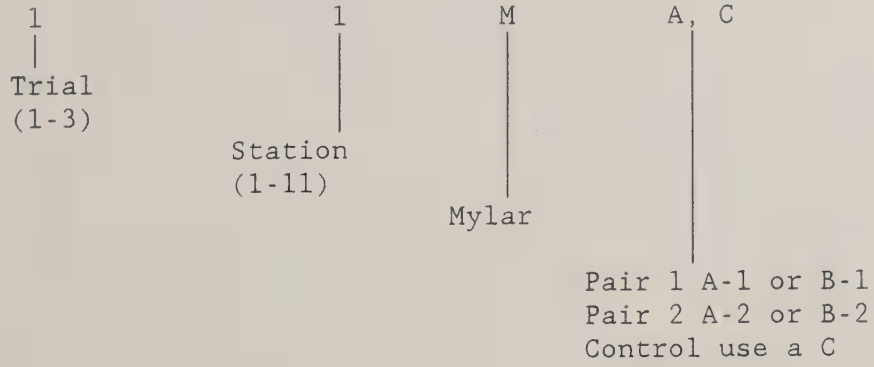
a. **Rotorod**



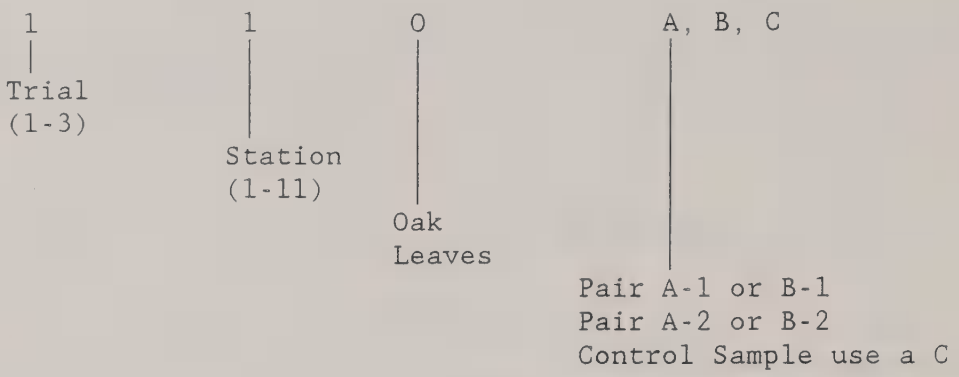
b. **Kromekote card**



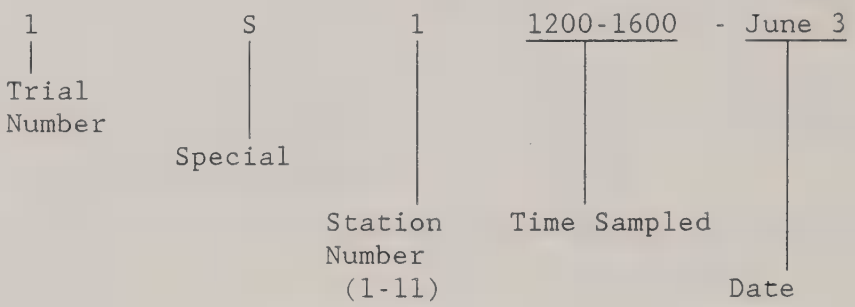
c. **Mylar Sheet**



d. Oak Leaves



e. **Special Dosage (Rotorod) Sampling**



Sampling Equipment Requirements

There will be 11 duplicate and paired sampling stations numbered 1-11 (Figure 2). The duplicates will be approximately at the same distance downwind but should be separated approximately 10 meters from each other where possible. DPG will provide all equipment and samplers except the Forest Service will provide Mylar, Kromekote cards, and card holders.

Equipment (Per Trial)

	Required	Controls	Spare
NICAD Battery	22	--	2
Rotorod motors w/bracket	44	--	10
Stakes (2" x 2" x 6') for Rotorods	44	--	22
Mylar card holders	44	--	22
Kromekote card holders	44	--	22

Samplers (Per Trial)

Rotorods spinning	44	5	22
Mylar sheet	48	5	22
Kromekote cards	44	0	22

Samplers and Equipment (Per Trial) for Special Dosage Sampling

NICAD Battery	1	-	1
Rotorod motors w/bracket	3	-	1
Rotorods	9	-	3

Special Dosage Sampling

In order to investigate the question of how long the Bt aerosol might reside over residential areas after treatment, we will operate Rotorod samplers at the mouth of Mill Creek Canyon. The samplers will be located at station 8 (Figure 1) and operated from 1200 hours of the treatment day through to 1600 of the following day. Three Rotorod samplers will be placed in a secured backyard of a resident near station 8. We have found that people are cooperative and helpful when they understand the purpose of the study and feel part of it. The Rotorods will be operated from 1200-1600 of each treatment day. Continuing they also will be operated from 1600 of the treatment day to 0800 of the following day, and 0800-1600 of the following day to provide a 24 hour sample (Table 3). Fresh Rotorods will be used for each sampling period. Samplers will be marked for identification as outlined under sampler marking paragraph. Forest Service personnel will handle these samples and DPG laboratory will do the assay.

Table 3. Schedule for special dosage (Rotorod) sampling.

Event	Sampling Period	Number of Rotorods
1.	1200-1600 treatment day	3
2.	1600-0800 treatment day extending to 0800 next morning	3
3.	0800-1600 day following treatment	3

Laboratory Assay

All samples will be kept under refrigeration until assay.

Rotorods. Rotorods will be retrieved from the motor by covering the Rotorod with a Ziploc bag and sealing the zip without directly touching the rod. Labels will be placed on the outside of the Ziploc bag and not on the Rotorod. In the DPG laboratory the Bt will be extracted from the Rotorod and the Ziploc bag, and the diluent will be diluted and plated. Collecting fluid will be retained for additional assay as required. Assay will produce colony forming units (CFU's) per Rotorod.

Kromekote cards. Kromekote cards will be retrieved after the drop stains have dried and placed in brown paper lunch sacks. They will be delivered to Harold Thistle, Project Meteorologist. Cards will assayed qualitatively for presence of Bt spray drop stains by the Forest Service.

Mylar Sheets. Mylar sheets will be retrieved in the field by sterile forceps and placed in sterile glass bottles or Ziploc bags. Bt will be extracted and plated-out in the same manner as the Rotorods at the DPG laboratory. Bt deposition could be high at those stations closest to the treatment block. Assay will produce colony forming units (CFU's) per Rotorod.

Oak Leaves. Gambel oak leaves will be retrieved at the same time as the Rotorods. They will be placed in a paper sack and stapled shut, and the sack marked as per instructions. Leaves will be assayed to produce CFU's per dry weight and unit area.

Laboratory data and Test Officer's report should be provided to Study Coordinator (Jack Barry) by August 1, 1993.

Field Data Requirements

Data requirements and person responsible for providing the data are listed below. These data will be needed for each of the three treatments of Block SL-2 Mill Creek.

1. Operational Treatment Data (R-4, John Anhold, Andy Knapp):

   Date
   Aircraft identification and description
   Pilot(s)
   Time block treatment began
   Time block treatment ended
   Total gallons applied
   Total acres treated
   Description of block treatment - specifically where the spray swaths were applied and when, provide this on a map that shows location of swaths.
   Remarks from pilot

2. Weather data from all meteorological instruments, Harold Thistle, Project Meteorologist. Specific needed immediately after each trial and:

   Mean Wind speed
   Mean Wind direction
   Mean Temperature
   Mean Relative humidity
   Cloud cover
   Barometric pressure (from National Weather Service, Salt Lake City airport)

3. Sampling (DPG, Project Officer)

   Time samplers set out
   Time samplers picked-up
   Time samplers were activated
   Time samplers were deactivated
   Time control samplers were activated, and deactivated, set out, and picked up
   Sampler malfunctions

4. Laboratory data (DPG, Project Officer)
   Total colony forming units (CFU) per each Rotorod and Mylar.
   Total CFU per gram dry weight of Gambel oak leaves.

5. Spray tank samples (Utah, Mark Quilter)

   Date
   Batch number

Introduction

The effects of Bt on non-target Lepidoptera will be examined in the laboratory while feeding field treated host material. This will be done as compared to field testing to minimize additional mortality factors that are associated with rearing larvae in the field. Host material will be placed at field sites which will be spaced down canyon from treatment area and exposed to different dosages of Bt through drifting. Many of the procedures and methods have not been tested, thus this work is in part a feasibility study to develop methodology for impact studies.

The application, spray material and meteorology support will be the same for this portion of the study plan as mentioned earlier in the Deposition/Dosage Sampling methods. Also, measures to avoid contamination will be similar to those mentioned earlier in the previous methods section.

Acquisition of Eggs and Larvae:

If available, eggs or larvae will be acquired for 6 species of locally rare Lepidoptera. Other sensitive Utah species will be used as a back-up if eggs or larvae are more readily available for these than for the 6 listed species. For the butterfly species Callophrys spinetorum, Callophrys sheridani and Incisalia fotis, eggs will be taken from wild-caught females. For I. fotis if adequate numbers of eggs are not taken from females, larvae will be collected from Cliff rose using the beat-sheet method. Also, if eggs are available through suppliers, they may be purchased. For Neominois ridingsii eggs will probably not be available by spring or early summer from any location within its range. For this reason, an attempt will be made to collect larvae in the wild and/or larvae will be purchased if a supplier can be found. For the moth species Catocala briseis and Catocala ophelia eggs will be purchased, if a supplier can be found. This is the only method of acquiring these two moth species for 1993.

For the appropriate species, eggs will be acquired from females by confining them in screened or clear plastic-sided laying cages of dimensions 6 x 6 x 6 inches. Females will be collected in the wild, placed in glassine envelopes, and stored in a cooler until the return to the laboratory and their release into laying cages. In the laboratory, the various species will be caged with their appropriate larval food-plants. Up to 8 females of a species will be confined to a cage. A 100 watt light bulb will be mounted approximately 6 inches above each cage to provide an optimal temperature (around 27° C) to induce ovipositing. Light bulbs will be controlled by a timer so that females will not be exposed to intense light for continuous 24 hour periods. Females will be fed daily by providing honey water in a small plastic 1/4 inch deep plastic cap fitted with a small sponge to absorb the honey water. A small piece of window screen will be fitted over the cap to prevent the butterfly's body or wings from contacting the sticky honey. Larval food-plants will be replaced with new clippings as needed to maintain freshness. Eggs will be collected from the cages when necessary. Since eggs

of Lycaenids are minute and very difficult to locate on the food-plants, 2 1/2 to 3 power reading glasses (available at Smith's Grocery Stores) will be worn when searching for and handling eggs. Collected eggs will be kept in small clear plastic Solo^{tm} Brand cups used by fast-food chains for various condiments like catsup or mustard (available at restaurant supply stores). For adequate statistical analysis, the desired number of larvae per species would be about 200, at minimum. The above procedures will be conducted at a Utah Valley College laboratory.

Rearing procedures:

During the project, workers will strictly adhere to standard Lepidoptera rearing procedures (Carter and Feeny 1985). Rearing will be done in clear plastic containers of various dimensions. As eggs hatch, and larvae become about 2nd instar they will be removed from Solo^{tm} cups to sandwich size clear plastic rearing containers containing fresh sprigs of the appropriate plant. Rearing will be done such that egg hatch and larvae development will as closely as possible represent that of the natural wild population. This will help simulate the impacts that would naturally occur at that larval stage. After the experimental part of the study begins, there will be 10 larvae per container. If extra larvae are obtained, they will be maintained as a reserve stock in larger containers each containing variable numbers of larvae. Fresh larval food-plants will be maintained in bottles or in florist's Aquapics for larvae to feed upon. These will in turn be placed in the clear plastic rearing containers. When plants need changing all larvae will be removed from old plants and placed on new ones. To avoid injuring small delicate larvae when handling, an artist's camel-hair paintbrush or a round toothpick that has been dampened will be used. Frass will be removed from containers and containers will be wiped down with 5% sodium hypochlorite solution on a weekly basis, or more frequently as needed.

Experimental design:

To quantify the effects on non-targets of varying amounts of Bt - varying amounts due to natural spray drift from the treatment block - two groups of 5 or more species of sensitive Lepidoptera will be established; a control group A and a treatment group B. There will be one host food-plant for each the 5 species at each of the 3 to 5 locations. Both groups will be reared in the laboratory under identical conditions except that group B will be fed foliage that is treated with Bt during field operations. The species to be used and their larval food-plants are;

Lepidoptera species	Larval food-plant
Callophrys spinetorum	various species of mistletoes on conifers.
C. sheridani	Shortstem buckwheat (*Eriogonum brevicaule*)
Incisalia fotis	Cliff-rose (*Pershia mexicana*)
Neominois ridingsii	Bluebunch wheatgrass (*Elymus spicatus*)
Catocala briseis	various Poplars
C. ophelia	various Poplars

Other sensitive species may be used if necessary and depending upon their availability. They are as follows in priority of potential availability:

Alt. Lepidoptera species	Larval food-plant
Satyrium saepium	Deer Brush (Ceanothus velutinus)
Thessalia leanira alma	Common Paintbrush (Castilleja chroma)
Satyrium californicaus	Chokecherry (Prunis virginiana)
Cyllopsis pertepida dorothea	Common violet
Boloria selene	

Sprigs of larval food-plants will be taken from 3 to 5 sampling sites located within and down-canyon (and downwind) of a Bt treatment plot in Millcreek canyon along the Wasatch Front. The locations of food-plant collection sites will rely upon existing knowledge of Bt drift rates (Barry and Teske 1992) such that plants at each site will receive different amounts of Bt., (see sample stations 1-5 of figure 1). If larvae become limiting, sampling stations furthest from the treatment area will be deleted first. Artificial Bt samplers will be located downwind of the treatment plot in order to record actual amounts of Bt drift at various distances from the treatment boundary. On the night before a Bt treatment, boughs or sprigs of the various larval food-plants will be placed in containers of water along the canyon bottom at the designated distances where artificial Bt samplers are located. At the sample station larval food-plant should be positioned perpendicular to the axis of the drainage winds adjacent to artificial samplers, figure 3. Since many of the species are woody types, they are expected to remain fresh for several days if kept in water. Following Bt treatment, sprigs will be taken from each larval food-plant at each sample site and placed in containers with 15-20 larvae of the appropriate larval species. Each container of larvae will be marked as to species and larval food-plant sample site. Before feeding sprigs to larvae however, samples of leaves (or flower pedals) will be taken from various locations on the bouquet which is to be fed so that the Bt contamination can be quantified per bouquet. Foliage samples will be placed in a paper sack and stapled shut, and the sack marked as described in sample marking section. Samples will be kept refrigerated until assay. At the Dugway Proving Ground assay lab Bt on the foliage will be quantified by cultivating the spores to yield the colony forming units (CFU's) per weight of dry plant material. When more plant is needed for any group B larvae, sprigs will be taken only from the appropriate sample site for that container of larvae. It is expected that boughs or sprigs placed in the canyon at the specific sampling sites will last at least 5 days before any drying takes place and some are expected to last for 15 days or more. Since there will be 3 Bt applications in the treatment plot, when necessary new larval food-plant will be placed in the canyon at sampling sites the night before the impending treatment. Some species of plant are expected to stay fresh through the 3 treatments (or up to a 15 day period). Freshly treated plant will be fed to group B larvae immediately following the 3 treatments. To maintain strict control of as many variables as possible, fresh untreated plant will be provided to group A larvae at the same time, whether their sprigs appear to need changing or not. There will be 20-30 larvae for each species for the control group A.

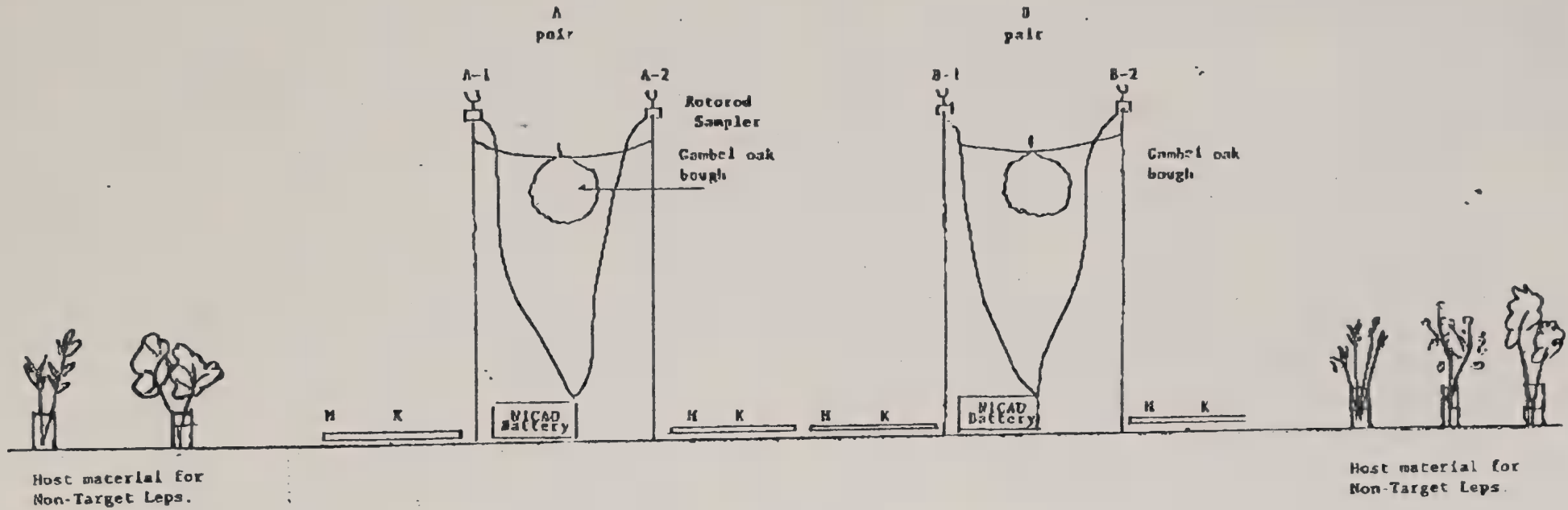
All dead larvae in either group will be collected and stored for future bioassays. Date of death will be recorded to the day of death as accurately as possible for both groups. For group B larvae the larval food-plant sample site will also be recorded. For the first treatment 15-20 larvae from each of the 5 lepidoptera species will be fed food-plant subjected to Bt from the 5 drift collection stations. These larvae will be fed food-plant subjected to all three treatment. In the second treatment, host food-plant will be fed to larvae of the first treatment, as mentioned above, and also to 15-20 larvae from each of the 5 lepidoptera species that have not yet been subjected to Bt sprayed foliage. And following the third treatment, host food-plant will be fed to the first and second treatment larvae and to 15-20 larvae of the 5 lepidoptera species not yet exposed to Bt. All larvae will be of the same age at the start of the experiment. Also, all remaining larvae following the first application will receive freshly treated foliage from a second and third application to determine the cumulative effect of Bt. After 3 Bt treatments, larvae will continue to be reared until those remaining either pupate or die. Also any subsequent pupae that die will be stored for bioassay. Accurate records will be kept for all deaths per species per sample site for group B. During all procedures above, care will be taken to prevent cross contamination between groups and between sample sites in group B.

Statistical Analysis

A survival analysis (Lee, 1992) will be used to compare the relationship between amount of Bt fed to larvae and mortality. The test will examine both cumulative effect of Bt over three treatments as well as thresholds of Bt required to cause mortality to specific Lepidoptera species.

Equipment List

Quantity	Item description
40	Quart sized or sandwich sized clear plastic rearing containers 1 package - Solo™ Cups
10	Clear plastic containers (quart sized) for reserve stock
50	Aquapics (various sizes)
10	Cages (for egg laying), dimensions (6x6x6 inches)
2	Camelhair brushes
6	Lamps and bulbs
1	Timer switch with multiple sockets
1	Magnifying reading glasses (2 1/2 or 3 power)



Notes:

* There will be 5 of these set-ups as shown, stations 1-5 of figure 1.

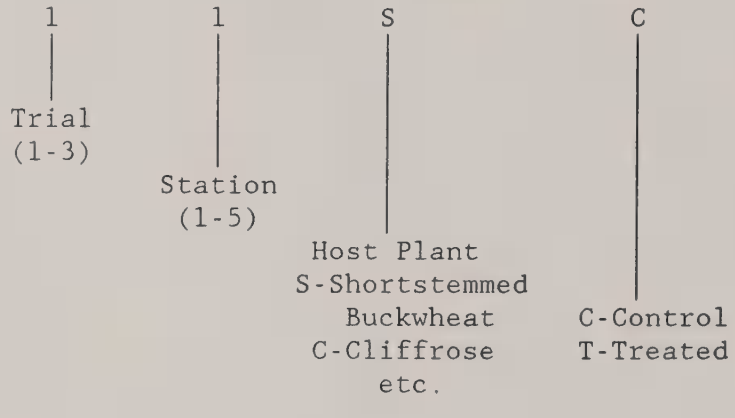
* Host material should be positioned perpendicular to axis of the drainage winds to avoid one sampler apparatus shielding the next.

Figure 3 - Diagram showing placement of host foliage for non-target Lipidoptera study, 1993.

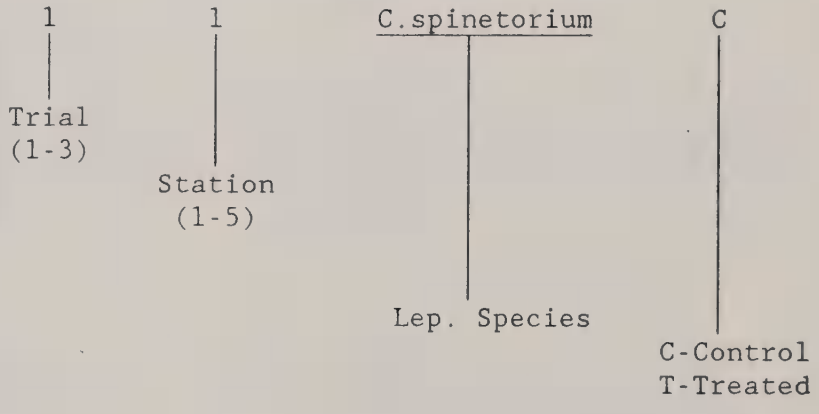
Host Material and Larval Sample Marking

Host material sample marking will be used throughout the study from the field to laboratory where samples are analyzed to the reporting of data. Codes will be marked on the outside of the paper sacks that contain leaf samples and on containers that have dead larvae. Date of mortality will also be recorded.

a. Host Plant Material



b. Lepidoptera Species - For Bio-Assay



DATA ANALYSES

Data analyses, initially, will follow each task, and as appropriate, integrate results and analyses. Data from the laboratory and field controls will be analyzed and considered in the analyses and discussed in the report.

Task 1 - to evaluate the effects of Bacillus thuringiensis on selected non-target Lepidoptera species in the Wasatch Mountain Range of northern Utah.

This will be done by Dr. Wayne Whaley. Selected non-target Lepidoptera will be fed host foliage that had been exposed to spray drift. Controls and indicator species will be used. Mortality/dose relationships will be investigated and evaluated statistically.

Task 2 - to quantify the deposition of Bt on host foliage of non-target Lepidoptera and to compare results with FSCBG model predictions of deposition on host foliage and insect mortality.

Bt CFU's observed on Gambel oak foliage will be compared to FSCBG model predictions and non-target Lepidoptera mortality resulting from feeding on Bt exposed host foliage. Statistical methods will be used to evaluate relationships of FSCBG deposition and dosage predictions.

Task 3 - to evaluate size of buffer zones needed to protect non-target Lepidoptera colonies from Bt spray drift.

Bt effects thresholds will be evaluated by feeding the selected non-target species with host foliage that had been exposed at various down-wing (canyon) locations. The Bt concentration on foliage should decrease with downwind distances from the treatment block. From this we hope to demonstrate a dose response curve and thus be in a position to recommend buffer zone widths.

Task 4 - to compare FSCBG model drift predictions to dosage recoveries on Rotorod samplers; and deposition recoveries from Mylar deposit samplers, and Gambel oak foliage.

Correlation coefficients and logarithmic least squares of FSCBG predictions to sampler recoveries will be explored to evaluate FSCBG predictions. This will be done for each of the 3 primary samplers.

Task 5 - to evaluate the ValMet module of FSCBG dispersion predictions by comparing predictions to Bt deposition and airborne dosage.

Using Bt as a tracer, deposition and dosage will be compared, as a function of downwind distance, to ValMet model predictions. ValMet predictions will also be compared to FSCBG predictions and statistically analyzed.

Task 6 - to evaluate relationship of Bt recoveries among sampler types and Gambel oak foliage.

Direct relationships between sampler types will be statistically explored and compared to non-target dose responses to corresponding Bt doses.

Task 7 - to investigate residual Bt aerosols that might persist at mouth of Mill Creek Canyon after treatment.

Data from each of the Rotorods expressed in total CFU's will be used to calculate dosage. The three sequenced sampling periods will give an indication of the time period when the Bt was in residence.

A statistician will be contracted to analyze these data and to assist in preparing the data analyses section of the report and manuscript. Relationship of recoveries among different sampler types will be evaluated.

COORDINATION

Treatment Supervisor - John Anhold (801) 476-9732
Responsible for overall conduct of the eradication program and providing manpower as requested.

Public Affairs Officer (PAO) - L.J. Western (801) 582-1984
Responsible for all public affairs activities to include press releases, media contacts, public inquiries related to the program and studies, and coordination with Melynda Petrie, US Army Dugway, PAO, (801) 831-2116.

Study Coordinator - Jack Barry (916) 758-4600.
Responsible for planning, coordination, documenting and reporting of the off-site spray movement study. He will also assay the Kromekote cards.

DPG Project Officer - Bruce Grim (801) 831-3371
Responsible for all coordination administration and support with U.S. Army Dugway Proving Ground to include coordination between PAO and DPG public affairs officer, other DPG support groups and DPG contractor. Responsibilities include coordinating data reporting, fiscal matters, and briefing field crews on test procedures and safety.

DPG Materiel Test Project Officer - Gary Sutton (801) 831-5638
Responsible for coordinating DPG field and laboratory support activities.

Project Meteorologist - Harold Thistle (406) 329-3981
Responsible for all meteorological operations for the SL-2 Mill Creek project, preparing meteorological data report, coordinating with other project meteorologists, and conducting analyses of Task 5. Provide GPS coordinates of sampler and meteorological instrument locations.

DPG Laboratory Officer - Lloyd Larsen (801) 831-5173
Responsible for preparation of samples, laboratory assay of samplers, quality control procedures, and reporting data.

Test Officer - (801) 831-5247
Responsible to DPG Project Officer for set-up operation, and pick-up of sampling equipment, inventory of equipment and samplers at pick-up and delivery, and quality control, prevention of sampler contamination, and reporting on field operations.

Utah State Liaison Officer - Mark Quilter (801) 538-7190
Responsible for liaison, coordination among State and local jurisdictions, study personnel; and providing tank samples.

Air Operations Officer - Andy Knapp (208) 364-4222
Responsible for providing aircraft spray operations data requested in paragraph 1 of Field Data Requirements.

Contractor

Responsible for conduct of the non-target Lepidoptera study.

SAFETY

Safety is everyone's responsibility both in practice and in reporting real and potential hazards. All personnel involved in this study will be familiar with and observe procedures outlined in the Operational Project Safety Plan and the DPG Safety Plan. Supervisors are responsible to insure that personnel read the Safety Plan and all personnel are responsible for safe work practices. The primary safety hazard is canyon driving, particularly access and egress; travel on unimproved roads; operating vehicles and equipment during early morning conditions; stopping along narrow canyon roads; electrical hazards from generators; and lifting of equipment. Weather stations will be located to avoid electrical wires and vehicle and foot traffic. Any use of bucket trucks or "cherry pickers" will first require a site inspection and clearance by the safety officer. Guy wires and stakes will be marked with fluorescent engineering tape. Weather stations may be fenced if safety and security is deemed to be a problem. The material safety data sheet and pesticide label for Bt are in the Appendix.

SECURITY

Project scientists will need to evaluate security requirements for each type and location of instrumentation, and coordinate with the treatment supervisor.

REPORTING AND TECHNOLOGY TRANSFER

Results of this test and the analyses will be reported in a USDA Forest Service data report. This will serve as a resource for preparation of papers, etc. Manuscripts may be prepared and submitted to Transactions of the Journal of Economic Entomology, Journal of Environmental Toxicology and Chemistry, American Society of Agricultural Engineering, and the Journal of Applied Meteorology; or to other suitable professional publication for possible publication. In addition, papers may be presented at other professional meetings as decided by project scientists. Applicable results will be distributed to USDA-FS, Forest Pest Management offices, and to cooperators. Findings will also be incorporated in training sessions directed at persons who develop environmental impact studies, and who plan and conduct aerial and ground spray operations. Bt data will be incorporated in pesticide background documents, EIS's and used at public meetings.

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1. Dipel 6AF Pesticide Label

2. Dipel 6AF Material Safety Data Sheet

3. Reprint - Collection Efficiency of Rotorod Samplers for Sampling Fungus Spores in the Atmosphere by Robert L. Edmonds

4. Rotorod Product Bulletin

5. Assay Procedures for Biological Simulants/Sampler Preparation (MT-L389, 2nd Revision, 20 October 1987)

6. Atomization - Droplet Distribution of Foray 48B

7. Test Officer Report

8. Field Crew Check Sheet and Report

APPENDIX 1

Dipel 6AF Pesticide Label

ABBOTT LABORATORIES

DiPel® 6AF

Biological Insecticide
Aqueous Flowable

NOT REGISTERED IN CALIFORNIA

Active Ingredient:

Bacillus thuringiensis, var. *kurstaki*, 10,750 International Units of Potency per mg (48 Billion International Units per gallon)	2.15%
Inert Ingredients	97.85%

EPA Reg. No. 275-59
EPA Est. No. 33762-IA-1

INDEX:

CAUTION:
KEEP OUT OF REACH OF CHILDREN

1.0 PRECAUTIONARY STATEMENTS

1.1 HAZARDS TO HUMANS

Avoid contact with skin, eyes or clothing. In case of contact immediately flush eyes or skin with plenty of water. Get medical attention if irritation persists.

2.0 DIRECTIONS FOR USE

It is a violation of Federal law to use this product in a manner inconsistent with its labeling.

Do not apply this product through any type of irrigation system.

3.0 STORAGE AND DISPOSAL

Do not contaminate water, food or feed by storage or disposal.

Storage: Keep containers tightly closed when not in use. Do not store at temperatures below 0°F or above 90°F. Roll or shake the drum before dispensing.

Pesticide Disposal: Wastes resulting from the use of this product may be disposed of on site or at an approved waste disposal facility. Do not contaminate water when disposing of equipment washwaters.

Container Disposal: Triple rinse (or equivalent), puncture and dispose of in a sanitary landfill, or by other procedures approved by state and local authorities.

4.0 GENERAL INFORMATION

DIPEL 6AF is a highly selective insecticide for use against listed caterpillars (larvae) of lepidopterous insects. Close scouting and early attention to infestations is highly recommended. Larvae must eat deposits of DIPEL 6AF to be affected. Always follow these directions:

- Treat when larvae are young (early instars) before the trees are extensively damaged.
- Larvae must be actively feeding on treated, exposed plant parts.
- Thorough spray coverage is needed to provide a uniform deposit of DIPEL 6AF at the site of larval feeding.
- Under heavy pest population pressure, use the higher label rates and/or consider a second application.
- If attempting to control a pest with a single spray, make the treatment when egg hatch is essentially complete, but before extensive crop damage occurs.
- An approved spreader-sticker may be added to diluted tank mixes to improve weather-fastness of the spray deposits. Do not add a sticker to undiluted product.

After eating a lethal dose of DIPEL 6AF, larvae stop feeding within the hour and will die within several days. Dying larvae move slowly, discolor, then shrivel, blacken and die.

DIPEL 6AF is completely water miscible and may be applied through conventional ground or aerial equipment with quantities of water sufficient to provide thorough coverage of infested plant parts. The volume of water needed per acre will depend on weather, spray equipment and plant canopy type. It is recommended that DIPEL 6AF be added to water and not in reverse order. Fill the mix tank with the appropriate quantity of water excluding the anticipated volume of rinse water from containers. Start the mechanical or hydraulic agitation to provide moderate circulation before adding

DIPEL 6AF. Add the required volume of DIPEL 6AF to the mix tank or plane hopper and continue agitation. Then add rinsate from the original containers. If a spreader-sticker is recommended, add the required amount to the water prior to the addition of DiPel and agitate until uniformly suspended. Mild agitation is sufficient to maintain mixture suspension during loading and spraying. Do not mix more DIPEL 6AF that can be used in a 144 hour period.

CAUTION: Rinse and flush spray equipment thoroughly with water following each use.

DIPEL 6AF can also be applied undiluted from aircraft for control of Spruce Budworm, Hemlock Looper, Jack Pine Budworm, and Gypsy Moth. It is recommended that rotary or other atomizers be used to provide droplet Volume Mean Diameters (VMD) of 20-80 microns for needle conifers and 50-150 microns for deciduous hardwoods. After prolonged storage, undiluted DIPEL 6AF should be recirculated once to redistribute prior to use. During loading and spraying, agitation of the undiluted product is unnecessary and should be avoided.

5.0 APPLICATION INSTRUCTIONS

DiPel 6AF may be used to protect trees and shrubs such as in:

- Forests
- Residential, municipal, and shade tree areas.
- Recreational areas such as campgrounds, golf courses, parks, and parkways.
- Ornamental, shade tree, and forest nurseries.
- Shelterbelts, rights of way, and other easements.

5.1 APPLICATION RATE FOR FORESTS, TREES AND SHRUBS*

Pest	Oz/100 Gal/ Acre[1] (Ground Equip.)	Ounces/ Acre (Aerial[2] Applica.)	BIU'S/A[3]
Bagworm	11 to 21	11 to 21	4 to 8
Blackheaded Budworm	21 to 32	21 to 32	8 to 12
Browntail Moth	21 to 54	21 to 64	8 to 20
California Oakworm	11 to 21	11 to 21	4 to 8
Douglas Fir Tussock Moth	21 to 43	21 to 43	8 to 16
Eastern Pine Looper	32 to 43	32 to 43	12 to 16
Eastern Tent Caterpillar	11 to 21	11 to 21	4 to 8
Elm Spanworm	11 to 21	11 to 21	4 to 8
Fall Webworm	11 to 21	11 to 21	4 to 8
Forest Tent Caterpillar	21 to 43	21 to 43	8 to 16
Fruittree Leafroller	11 to 21	—	4 to 8
Green Striped Maple Worm	21 to 32	21 to 32	8 to 12
Gypsy Moth	21 to 107	21 to 107	8 to 40
Hemlock Looper	32 to 43	32 to 43	12 to 16
Jack Pine Budworm	21 to 43	21 to 43	8 to 16
Mimosa Webworm	11 to 21	—	4 to 8
Oak Leaftier	21 to 32	21 to 32	8 to 12

5.1 APPLICATION RATE FOR FORESTS, TREES AND SHRUBS* (cont.)

Pest	Oz/100 Gal/ Acre[1] (Ground Equip.)	Ounces/ Acre (Aerial[2] Applica.)	BIU'S/A[3]
Pine Butterfly	21 to 32	21 to 32	8 to 12
Redhumped Caterpillar	11 to 21	11 to 21	4 to 8
Saddleback Caterpillar	11 to 21	—	4 to 8
Saddled Prominent Caterpillar	11 to 21	11 to 21	4 to 8
Spring & Fall Cankerworm	11 to 21	11 to 21	4 to 8
Spruce Budworms[4]	11 to 80	11 to 80	4 to 30
Western Tussock Moth	11 to 21	11 to 21	4 to 8

* Forest, shade, sugar maple, trees and shrubs.

[1] Water dilution rate for hydraulic sprayer may be varied depending on coverage. For mist blowers, mix the applicable amount (oz.) in up to 10 gallons of water.

[2] For diluted aerial application, use in up to 10 gallons of water depending on type and density of trees. For best results spray systems which deliver droplet size of 150 microns VMD, or less should be used. NOTE: For Hemlock Looper and Eastern Pine Looper use 1-2 applications, undiluted, beginning at peak first instar. When applying two applications, apply each application at a recommended rate of 32 ounces/A. First application is applied at peak first instar and second application is at second instar. For the high rate, i.e. 43 ounces/A, apply single application only at first instar.

[3] Billion International Units per acre.

[4] Use rates greater than 21 ounces in Northern states for heavy populations.

6.0 NOTICE TO USER

Seller makes no warranty, express or implied, of merchantability, fitness or otherwise concerning use of this product other than as indicated on the label. User assumes all risks of use, storage or handling not in strict accordance with accompanying directions.

Abbott Laboratories – Quality Health Care Worldwide
Agricultural Products, North Chicago IL 60064 (800) 323-9597

APPENDIX 2

Dipel 6AF Material Safety Data Sheet

PRODUCT NAME:DiPel(R) 6AF

REACTIVITY

Incompatibility: Alkalinity inactivates product

Hazardous Decomposition or By-products: N/D

Conditions to Avoid: N/D

HEALTH HAZARD DATA

Routes of Entry: Inhalation - N/D Skin - No Ingestion - No

Oral Toxicity: LD50 > 5,000 mg/kg in rats. No signs of toxicity were observed

Dermal Toxicity: LD50 > 2,000 mg/kg in rabbits. No signs of toxicity were observed

Inhalation Toxicity: N/D. Five human volunteers inhaled 100 mg of B.t. ssp. kurstaki per day for five days without ill effect. Inhaled B.t. organisms may remain viable until cleared by host defense systems but are not pathogenic

Corrosiveness: No

Dermal Irritation: Slightly irritating in a skin irritation test in rabbits; reversible within 9 days

Ocular Irritation: Irritant. Slightly to moderately irritating in an eye irritation test in rabbits; reversible within 7 days

Dermal Sensitization: Sensitizer. Considered to be a sensitizer in a skin sensitization study in guinea pigs

Special Target Organ Effects: None found in people. In subchronic oral toxicity studies in rats and sheep and a 2-year chronic oral toxicity study in rats, with DiPel(R) Technical Powder no significant treatment-related effects were observed. In a 4-week inhalation study in guinea pigs, animals receiving 0.2 mg/l DiPel (R) Technical Powder exhibited evidence of inflammation and reduced adrenal weights; two of twenty animals died (NOEL = 0.02 mg/l or 20 mg/m3

Carcinogenicity: NTP - NL IARC - NL OSHA - NL ACGIH - NL

Signs and Symptoms of Exposure: N/D. Data suggest skin or eye irritation and allergic skin reactions are possible. Inhalation may result in respiratory tract irritation

PRODUCT NAME:DiPel(R) 6AF

HEALTH HAZARD DATA (cont)

Medical Conditions Aggravated by Exposure: Pre-existing eye, skin or lung lesions

Emergency and First Aid Procedures: Remove from source of exposure. If skin or eye contact occurs flush with copious amounts of water. If irritation persists or signs of toxicity occur, seek medical attention. No known antidote. Provide symptomatic/supportive care as necessary

SPECIAL PROTECTION INFORMATION

Ventilation: Use local ventilation

Respirator: Approved dust/mist respirator (as required)

Gloves: Rubber

Eye Protection: Chemical goggles or safety glasses

Other Protection: N/D

SPECIAL HANDLING AND STORAGE

Special Precautions: Avoid excessive storage temperatures and direct sunlight (above 100F)

Spill or Release Procedures: Sweep up product, place into appropriate container for disposal. Avoid dust. Ventilate and wash spill area

Waste Disposal: Dispose of product in accordance with federal, state, and local regulations

Other Handling: N/D

PRODUCT NAME:DiPel(R) 6AF

Legend

N/A = NOT APPLICABLE
N/D = NOT DETERMINED
NL = Not Listed
L = Listed
C = Ceiling
S = Short Term
(R) = A registered trademark of Abbott Laboratories
(TM) = A registered trademark of Abbott Laboratories

dipel®

Bacillus Thuringiensis Insecticide

TOXICOLOGY PROFILE

TOXICOLOGY

As evident from toxicology results, Dipel is one of the safest insecticides in use today. Its active ingredient is a bacterium, *Bacillus thuringiensis* (*B.t.*), which occurs naturally in the environment. *B.t.* has a highly specific mode of action. It effectively controls caterpillar larvae; however, the HD-1 strain of *B.t.* used in the production of Dipel has shown no toxicity to mammals, fish or other wildlife at recommended field rates. This is supported by Abbott's full toxicologic evaluation of Dipel and extensive testing by independent scientists. Further, in over 10 years of commercial use, no reports of adverse effects to the environment have been documented. Unlike most chemical pesticides, Dipel is ideally suited for use in integrated pest management programs since the active ingredient does not interrupt activities of beneficial insects.

ORAL TOXICITY

No toxicity in mice, rats or dogs has been demonstrated with single dosages up to 10,000 mg/kg of body weight.

Thirteen-week dietary administration of technical material to rats at dosages of 8,400 mg/kg produced no toxic effects.

Two-year chronic dietary administration of technical material to rats at 8,400 mg/kg produced no tumorigenic or oncogenic effects.

INHALATION TOXICITY

No toxic effects were observed in rats when Dipel 4L was instilled directly into the lungs at rates up to 5 mg/kg of body weight. This translates to a value 10,000 times greater than a bystander could expect during spray programs. Humans exposed daily to *B.t.* spores for over 10 years have shown no adverse effects.

DERMAL TOXICITY

Mild, transient dermal irritation was seen, but no systemic toxicity was noted in rabbits when Dipel 4L was applied to abraded skin at 1 mg/kg/day for 21 days. In other studies, a single epidermal application of Dipel 4L at 7.2 g/kg was not toxic to rabbits.

EYE IRRITATION

No corneal opacity was observed in rabbits treated with 0.1 ml of Dipel 4L. Only mild, transient irritation was noted in this study, and in other tests with wettable powder formulations.

SENSITIZATION

No evidence of sensitization was noted in guinea pigs given repeated subcutaneous injections of *B.t.* technical material.

I.V. INJECTION

A single I.V. dose of 10^8 *B.t.* spores was not toxic to young growing rats. There was no evidence of sporulation of *B.t.* within the visceral tissues over the course of a 112-day experiment.

TOXICITY TO FISH

No adverse effects were shown in rainbow trout and bluegills exposed to *B.t.* technical material for 96 hours at concentrations of 560 and 1,000 ppm.

A small marine fish, *Anguilla anguilla,* was not adversely affected by exposure to 1,000-2,000 times the level of *B.t.* expected during spray programs.

Field observations, one month after aerial application of Dipel, revealed no effects on populations of brook trout, common white suckers and smallmouth bass.

TOXICITY TO ZOOPLANKTON

Aerial spraying at labeled rate of Dipel 4L had no effects on populations of Cladoura, Copepoda and Rotifera species.

TOXICITY TO BIRDS

LD_{50} — Bobwhite Quail — Greater than 10 grams *B.t.*/kg body weight; autopsy of the birds revealed no pathology attributable to *B.t.*

Field observation of 74 bird species revealed no population fluctuations after aerial application of Dipel.

TOXICITY TO BEES

No toxicity to honeybees has been demonstrated during extensive laboratory and field studies with Dipel products at labeled rates.

TOXICITY TO BENEFICIAL INSECTS

No toxic effects to beneficial or predacious arthropods have been observed at labeled rates of Dipel. These results are based on laboratory and field studies performed on over 200 species of beneficial insects/spiders in the orders: Hymenoptera, Diptera, Neuroptera, Orthoptera, Araneae, Coleoptera and Hemiptera. Due to its safety to beneficials and unique mode of action, Dipel is an ideal component of integrated pest management programs.

RESIDUES

Since Dipel products have not been shown to be toxic to nontarget organisms, spray drift and residues do not present a health hazard.

TOLERANCE

Dipel has been granted exemption from the requirement of tolerance on all registered crops in Canada and the United States. The wettable powder formulation may be applied to certain raw agricultural commodities after harvest.

VIRAL ENHANCEMENT

The susceptibility of cell cultures to viral infection was not enhanced after Dipel 4L exposure.

WORKER SAFETY

People mixing and applying Dipel need not wear protective clothing.

ABBOTT LABORATORIES
Chemical & Agricultural Products Division
North Chicago, Illinois 60064, (312) 937-5100

October, 1982

Printed in U.S.A.

AG-3238

Fact Sheet

Bacillus Thuringiensis (Bt)

March 1992

United States Department of Agriculture

Animal and Plant Health Inspection Service

What is Bacillus thuringiensis?

Bacillus thuringiensis (Bt) is a naturally occurring bacterium that the U.S. Department of Agriculture (USDA) uses to control caterpillars. This particular strain of Bt is toxic only to insects in the order Lepidoptera in their larval stage. This order of insects includes the spruce budworm, tent caterpillar, spring and fall cankerworm, cabbage looper, and gypsy moth--all damaging agricultural pests.

Bt has a proven safety record and has been used in gypsy moth control strategies since the 1950's in the eastern United States, and the States of Washington and Oregon.

How Bt Works

Found in the soil, Bt is the natural cause of death for caterpillars that eat the bacterium. After eating Bt-treated foliage, caterpillars stop feeding and their movements slow down. Generally they die of starvation or bacterial infection within 7 to 10 days.

Who uses Bt?

Because Bt occurs naturally in nature, it is not harmful to the environment. For this reason, the USDA's Animal and Plant Health Inspection Service (APHIS) and the Forest Service (FS) often prefer its use over alternative methods of caterpillar control. Both APHIS and FS are using Bt for control and eradication of the European gypsy moth in forested lands in the northeastern United States.

Foresters, farmers, and gardeners use more than 3 million pounds of Bt every year, making it the biological pest-control material used most frequently in the United States. Three products--Dipel, Foray, and Thuricide--are currently on the market for control of gypsy moth caterpillars.

Even though Bt is a valuable tool when it is used to protect crops or forests against the gypsy moths, it is not a panacea. It is a readily biodegradable natural product that loses its effectiveness a few days after application. When pest control workers apply Bt against the gypsy moth, they must use several applications and time them to coincide with the emergence of newly hatched caterpillars.

With the development of integrated pest management techniques and concern for environmentally safe pest control methods, it is likely that Bt and other biological control agents will become more and more a part of insect-control programs in government and in the private sector, in both large commercial farms and backyard gardens. Bt is commonly used by organic growers and is approved for use on more than 200 crops.

Recommended Practices During an Eradication Program

Even though Bt is one of the safest pest control substances available on the market, it is reasonable for individuals to minimize exposure to any materials of this nature. Individuals in spray areas, especially those with immuno-compromised conditions, should:

1. Remain indoors for at least 10 minutes following the spray application.
2. Wait until moisture from the spray and dew has dried on the grass/shrubs before allowing children to play outside. Encourage hand washing.
3. If contact is made with the spray, wash the affected skin with soap and water. If the material should get into the eyes, flush with water.

In some situations, individuals may be unable to follow these recommendations. Human risk assessment data indicate that inadvertent exposure to Bt spray will not cause any detrimental health effects. The above recommendations are made to assure that exposure will be minimized to the greatest extent possible, with minor inconvenience. People with concerns about the exposure of their immune systems to Bt should contact their physicians for specific advice.

How Scientists Discovered Bt

Scientists have known about Bt since early in the 1900's. In the beginning of the century, people observed massive caterpillar die-offs in silkworms and in pests of stored grain. In studying why the die-offs happened, scientists discovered in the caterpillars a

rod-shaped bacterium called a bacillus. The disease-causing bacillus was named thuringiensis by a microbiologist named Berliner, after his native Thuringia, in central Germany.

Experiments to Perfect Bt

It didn't take long for scientists to begin experimenting with Bt as a natural insecticide by spraying it on foliage favored by attacking caterpillars. However, early attempts to apply Bt as a natural insecticide produced erratic results.

Bt--and certain other bacteria that affect insects--contain small crystals and reproductive spores. Both spores and crystals must be present in balanced numbers for the material to work effectively against some insects. However, the need for this balance was not discovered until after many years of research.

Beginning in the 1930's, French scientists developed ways to standardize and measure potency of Bt formulations. This discovery made manufacture of Bt feasible and results of treatments more uniform. In the late 1960's, USDA scientists developed improved cost-saving techniques for the fermentation process used to mass produce the material. In 1970, a strain of Bt was developed that greatly improved its potential as a natural insecticide. Mass quantities of this strain have been used since the mid-1970's.

Recent Research

State, federal, and industrial scientists continue to conduct research on thousands of Bt strains--some of them effective against insects other than caterpillars. Recently, this research has led to development of Bt strains effective against blackflies and mosquitoes.

Reprint - Collection Efficiency of Rotorod Samplers for Sampling Fungus Spores in the Atmosphere by Robert L. Edmonds

COLLECTION EFFICIENCY OF ROTOROD SAMPLERS FOR SAMPLING FUNGUS SPORES IN THE ATMOSPHERE

Robert L. Edmonds [1]

Abstract

In the sampling of fungus spores in the atmosphere, the collection efficiency, and thus the accuracy of the samplers for obtaining quantitative data, has rarely been considered for the particular fungus spore being sampled. This paper is designed to make potential users of Rotorod impaction aerosol samplers aware of the importance of considering sampling efficiency. A method for calculating efficiency is given.

Adequate study of the dispersion of fungus spores requires accurate sampling of the atmosphere. In many investigations the collection efficiency, and thus the accuracy of samplers, has not been considered for the particular type of fungus spore being sampled.

Suction devices and rotating arm impactors are the most common types of instruments used in the collection of fungus spores. Because of the large size range of fungus spores (from a few microns (μ) to 100μ), however, there is no one instrument yet developed that is capable of sampling the whole range with equal efficiency.

This paper proposes to indicate to users of rotating arm impaction samplers the importance of considering collection efficiency in sampling; to discuss theoretical aspects in determination of efficiencies; to provide an equation for determination of sampling efficiencies for various sizes of fungus spores; to demonstrate how collecting surfaces can be modified to in-

[1] The author is presently Program Coordinator, United States International Biological Program, Aerobiology Program, Botany Department, University of Michigan, Ann Arbor, Michigan 48104.

crease sampling efficiency; and to discuss the importance of the selection of a suitable sticky material for the leading edge of the sampler. The "Rotorod sampler" is used as an example of this type of device. It is commercially available and widely used.

The "Rotorod sampler" in the form developed and marketed by Metronics Associates, Inc. of Palo Alto, California (10) has been used for collecting fungus spores in the atmosphere by many workers including Asai (1), Froyd (5), Barksdale (2), Skilling (9), and Edmonds (4). It employs the process of inertial impaction with spores, and so forth, being impacted on a whirling arm.

Advantages of this sampler are low cost, simplicity, light weight, large sampling volume, suitability for experiments employing large numbers of simultaneous samplers, battery operation for remote locations, and the collection efficiency is not affected by wind speed up to 6.2 kph. The chief disadvantage is that collection efficiency is sharply dependent on spore size and density, and it can only be used for short periods of time because of over-loading of the collection surface.

Two sizes of Rotorods are available commercially from Metronics (Fig. 1). The U-shaped brass Rotorod has collection surfaces 1.59 mm in thickness, with arms 6 cm high, 8 cm apart and samples 120 liters per minute (lpm). It was designed to sample particles in the 15-25μ diameter range. The H-shaped chromel Rotorod is 0.38 mm in thickness, with arms 6 cm high, 12 cm apart and samples 41.3 liters per minute. It was designed to sample fluorescent particles (specific gravity 4.0 g cm^{-3}) in the 1-5μ diameter range (10). The Rotorod motors revolve at approximately 2400 rpm, with the collecting surfaces of the U- and H-shaped Rotorods revolving at 15.1 and 10.1 m sec^{-1}, respectively. The actual rpm for each motor varies and is supplied for each motor.

FIGURE 1. Rotorod samplers and motors. Commercially available Rotorods are the U-shaped (right) and H-shaped (center). Modified Rotorod is on the left.

DISCUSSION

Most researchers who are interested in collecting airborne spores usually wish to know the spore concentration in the atmosphere. Erroneous spore concentrations, however, can be calculated if collection efficiencies are not considered for the particular spore size under consideration. Not all spore sizes, even within the suggested range of the instrument, are collected with equal efficiency. Sampling sensitivity is low if efficiency is low, and low efficiencies also result in uneven distributions of spores on the collection surfaces (8). This is important to consider if sample fields are to be counted on the Rotorod arms.

Noll (8) has discussed theoretical and experimental aspects of whirling arm samplers. The collection efficiency (E) is largely a function of the particle parameter (P) (Fig. 2). Experimental data in this figure were obtained from a 16-stage rectangular collector impaction sampler developed by Noll. Each stage was designed to sample a specific size range of particles at 85-100% efficiency. The particular data used were derived from two stages designed to collect particles down to 26 and 13μ respectively. These stages had collection surfaces 3.2 and 0.8 mm in width, respectively, and revolved at 7.2 m sec^{-1}. Noll's data generally agree with experimental data of Chamberlain and Gregory for impaction of Lycopodium spores on cylinders (3). The efficiency suggested by Chamberlain for P equal to 10, however, is lower than those values suggested by Noll. Noll's data are preferred in practice because they are derived from a whirling arm sampler similar to the Rotorod, with similar sized rectangular collection surfaces, revolving at similar velocities. The line through the data was drawn as the line of best fit by eye.

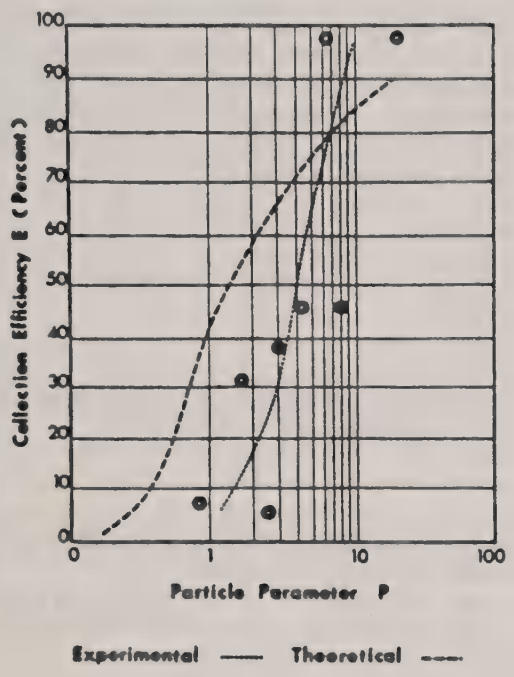

FIGURE 2. Relationship between particle parameter (P) and spore collection efficiency (E). (After (8)).

The theoretical curve presented in Figure 2 was determined by Langmuir and Blodgett for flow around a ribbon (8). Experimental values of E are lower than theoretically derived values, for P less than 7, but higher for P greater than 7. Chamberlain noted that experimental values of E were always lower than theoretical. He is uncertain whether this represents a fault in theory or a failure by sticky cylinders to retain all spores striking them. It would appear that the desired efficiency of 100% is approached as P approaches values of 10 or greater. In practice, because of the inconsistent agreement with theory, it is preferable to use the experimentally derived curve to determine E.

The selection of a suitable sticky material for the leading edge is important. If the surface is dry, particles bounce off. The material must be sticky, but if it is too thin, friction causes it to run off. If it is too thick, the edge loses its sharpness, the effective size of the collection surface is increased, and the collection efficiency is lowered. A 1:3 rubber cement and xylene solution used by Harrington, et al. (7), Froyd (5), and Edmonds (4) appears to give reasonable results.

The following is a general formula to calculate P for any spore size:

$$P = \frac{V_o \, d^2 \delta_p}{18 \, \eta \, L \, S}$$

P = Particle Parameter (dimensionless)
V_o = Average* Rotorod arm velocity (cm sec^{-1} U-shaped (1010)
H-shaped (1510)
d = Diameter of sphere of equivalent volume to that calculated for the spore (cm)
δ_p = Density of spore (g cm^{-3})
η = Viscosity of air (poises, g sec^{-1} cm^{-1}), at 18°C = 182.7 x 10^{-6} poises
L = Width of rectangular collector (cm)
S = Dynamic shape factor of particle (dimensionless)

*Actual arm velocity is variable because rpm vary from motor to motor.
Actual rpm for each motor is supplied by manufacturer.
The value of E is read from Figure 2.

Fuchs (6) has suggested that S is 1.28 for ellipsoids with ratio of axes, major/minor = 4. For practical purposes, no great error is made by setting S = 1 for spores with ratio of axes less than 4.

Table 1. Values of particle parameter (P) and collection efficiency (E) of U-shaped Rotorods in spore sampling studies.

Author	Organism	Type of spore[a]	Average dimensions of spore (μ)	Diameter of spherical spore of equivalent volume (μ)	P particle parameter (from formula)	E Percent (from Figure 2)
Asai (1)	*Puccinia graminis*	Uredospore	24 x 18.5	22	9.4	95
Froyd (5)	*Hypoxylon pruinatum*	Ascospore	26 x 10.5	19	7.0	80
Barksdale (2)	*Piricularia oryzae*	Conidia	23 x 8.5	16	4.9	65
Skilling (9)	*Scleroderris lagerbergii*	Ascospore	19.5 x 5	12	2.8	30

[a]Spore density was assumed to be 1.0 and S = 1 (ratio of axes of spores is less than 4). Diameter of equivalent sphere is rounded to nearest whole number.

Asai, Froyd, Barksdale, and Skilling used U-shaped Rotorods in their experiments. Asai, however, was the only investigator to mention sampling efficiency, noting that spores in the vicinity of 20μ diameter are impacted at approximately 100% efficiency.

Table 1 shows values of P and E for each of the four studies. The *Puccinia graminis* spores trapped by Asai are impacted at close to 100% efficiency. The other spores are sampled at much lower efficiencies. Unit densities for spores was assumed.

The H-shaped Rotorods will impact spores of unit density down to 9μ in diameter at close to 100% efficiency. Thus, in selection of Rotorod size to be used, it is important that sampling efficiencies for both sizes of Rotorods be determined for the particular spore in question, in order to obtain maximum efficiency. If P is greater than or equal to 10, the efficiencies close to 100% can be obtained.

H-shaped Rotorods can also be modified (Fig. 1) to a particular efficiency by welding aluminum shims of different widths to the arms, thereby adjusting the width of the collection surface and increasing the collection efficiency. Welding of shims to arms increases the aerodynamic drag on the rotating arms, which probably results in small decreases in the rotation speed of the sampler perhaps of 5% or so. This was not checked in practice, but it should be considered. This modification shown in Figure 1 was used by the author to collect spores of *Fomes annosus* (4.5-5.0μ diameter)(4) at an efficiency similar to that of fluorescent particles (specific gravity 4.0 g cm^{-3}, 3.0μ average diameter) collected on H-shaped Rotorods. This enabled a direct comparison of their respective dispersion patterns, with the object of determining if fluorescent particles could be used for tracing spore dispersal.

Another factor to be considered in collection efficiency is that of possible changes in shapes and, thus effective sizes of fungus spores while they are airborne due to changes in moisture content, and so forth. This is difficult to assess and thus has not been considered in the calculations.

CONCLUSION

In sampling fungus spores with a rotating arm impaction device such as the Rotorod, it is important to consider the collection efficiency for the particular species of fungus spore being sampled in order to make accurate calculations of the concentration in the atmosphere. If the particle parameter P is calculated to be greater than 10, then the efficiency of collection is close to 100%. If P is less than 10, then the collection efficiency can be read from Figure 2.

An alternative to this is to modify the size of the collecting surface to increase collection efficiency.

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A QUANTITATIVE SAMPLING METHOD FOR AIRBORNE SWEET CORN POLLEN UNDER FIELD CONDITIONS[1]

P. K. FLOTTUM, D. C. ROBACKER, AND E. H. ERICKSON, JR.[2]

Abstract

The rate of pollen dehiscence in a sweet corn (*Zea mays* L.) plot was measured using a Rotorod Sampler®. Samples were taken from 0700 to 1230 h for 3 days during anthesis. Totals were averaged over the 3 days, and the resulting composite data were used to develop a sampling protocol accurate for determining the rate of pollen release. Results showed that a 10-min sampling period, with a frequency of at least once every half hour was required to accurately reflect the pollen release rate.

Additional index words: **Pollen, Dehiscence, Pollen emission profile, *Zea mays* L.**

STUDIES of pollen dehiscence in sweet corn (*Zea mays* L.), and many other grasses (Gramineae) have been primarily concerned with developing techniques that predict the date flowering will begin (Cross and Zuber, 1972; Gardner et al., 1981; Hanway 1966). In sweet corn the process of pollen dehiscence, usually defined only as anther decention, is fairly well understood (Knox, 1979; Percival, 1969). However, studies of patterns of pollen release for a single day or for the period of anthesis have generally been qualitative in nature, as quantitative in vivo measurements of airborne sweet corn pollen have not been made.

This paper 1) describes a method used to measure the pollen emission patterns in a flowering sweet corn field, 2) documents the effectiveness of the method, and 3) presents data pertinent to the optimal use of the method.

Materials and Methods

A Rotorod Sampler®[3], a rotating impaction device powered by a 12 v battery, was used to collect airborne sweet corn pollen. Airborne pollen is captured on the leading surface of removeable 64-mm plastic rods held by the rotating arms (Fig. 1). General Electric G-697 Silicone Grease®[3] facilitates capture and retention of the pollen grains. The arms are rotated at ca 2400 RPM, sampling a volume of ca. 120 L/min. Operating efficiency, or ability to collect airborne pollen in the volume sampled, was determined to be greater than 99% by manufacturers specifications.

These studies were conducted at Madison, Wis. during 1981. The Rotorod Sampler was positioned centrally in a 40 m² plot of 'Commander' sweet corn with the maximum height of the sampler rods slightly below center of representative tassels in the plot. Sample rods were replaced at the end of each sampling interval whereupon the number of captured pollen grains were counted.

Two concurrent collection procedures were designed and

Fig. 1. The Rotorod Sampler®, showing removable 64 mm plastic rods.

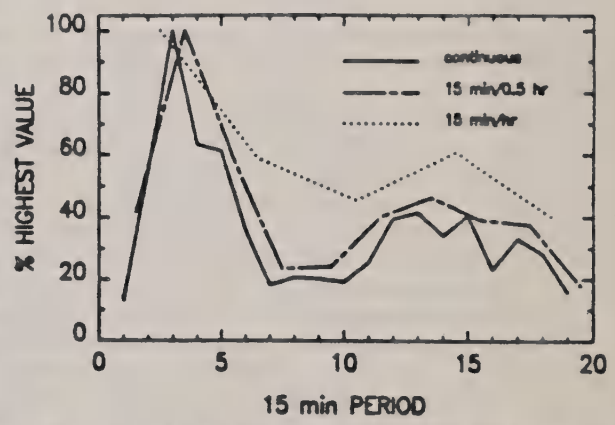

Fig. 2. Relationship of continuous, 1 sample/h and 1 sample/0.5 h profiles.

their results compared. The first procedure, subsequently referred to as the continuous method, consisted of running one Rotorod Sampler for 22 consecutive 15 min periods, from 0700 to 1230 h, for 3 days in the flowering sweet corn plot. Actual numbers of collected pollen grains were converted to pollen grains/L and plotted by period to develop a pollen emission profile for each day. The three daily profiles were then aligned so that the 15-min periods containing the initial daily peak coincided. This was done in order to accommodate the relative times of pollen dehiscence, not absolute time of day. Initial daily samples containing no pollen were not included in the analysis. Thus, the resulting composite profile consisted of 19, 15-min periods (Fig. 2).

To determine if reliable data could be obtained from fewer sampling periods than the 19 used to develop the composite of the continuous sampling method, the same data were reanalyzed assuming 1) one 15-min sample/h and; 2) one 15-min sample/0.5 h. For the one sample/h method, three groups of 15-min periods were analyzed as

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[3] Ted Brown Assoc., 26338 Esperanza Dr., Los Altos Hills, CA 94022. Mention of a trade name does not constitute a guarantee or warranty of the product by the USDA nor an endorsement over other products not mentioned.

if each group represented a distinct replication of the method. From Fig. 2, nonrandomized groups used were:

Group 1: periods 1,5,9,13, and 17
Group 2: periods 2,6,10,14, and 18
Group 3: periods 3,7,11,15, and 19

For the one sample/0.5 h, two nonrandomized groups were analyzed as distinct replications:

Group 4: periods 1,3,5,7,9,11,13,15,17, and 19
Group 5: periods 2,4,6,8,10,12,14,16,18, and 19[4]

Regressions of pollen emission on period were conducted for each of the three data arrangements (continuous, one 15 min sample/h and one 15 min sample/0.5 h) using orthogonal polynomials to represent period numbers.

The second collection procedure used consisted of running another Rotorod Sampler® for a period of only 10 min/sample. Samples were collected for the first 10 min of periods 1,3,5,7,9,11,13,15,17, and 19, (one 10 min sample/0.5 h), with the sampler left idle for the remaining 5 min of the period. These samples were neither randomized nor replicated. Results of these 10-min samples were analyzed as above and compared to the composite results and to the results of the one 15-min sample/0.5 h.

Results and Discussion

Method 1

Daily pollen emission profiles were strikingly similar. A large peak was recorded during one of the first 3 15-min periods each day. This was followed by a reduction in emission intensity, another slight increase then decreasing thereafter.

Regression analyses to determine the relationship between the amount of pollen collected and the time of day demonstrated significant linear ($P < 0.05$), quartic ($P < 0.01$), and quintic ($P < 0.05$) coefficients for each of the three daily profiles and the composite.

Regression coefficients for the one sample/0.5 h method, and for the composite profile (continuous method) were not significantly different (linear = -0.01 vs. -0.01; quartic = -0.004 vs. -0.002; quintic = 0.005 vs. 0.001, respectively - regression coefficients not converted from orthogonal polynomials). Similar analyses for the one sample/h method showed linear significance ($P < 0.05$), but neither the quartic nor the quintic coefficients were significant. Therefore, by inspection and analyses interpretation, it is shown that the curves for the continuous and one sample/0.5 h data arrangements are clearly high degree polynomials, while the curve for the one sample/h data arrangement is only a first degree relationship. These relationships are represented graphically in Fig. 2. Had sampling begun earlier each day, the initial peak may have been evident in the one sample/h arrangement, but this would not alter the lack of significance for the second peak.

Method 2

Results of the second technique, running the sampler for a period of 10 min/0.5 h, were compared to the results of the continuous method and to those of the one 15 min sample/0.5 h data arrangement. These were not significantly different at the levels previously noted as there were no differences in the rate of pollen collection or shapes of the profile curves. Hence, one 10 min sample/0.5 h retained the accuracy of continuous sampling and displayed the bimodal emission profile of the sweet corn population. Further, this sampling frequency optimized accuracy of the data and reduced the effort required in data acquisition. Moreover, it permits one operator to gather data simultaneously from several locations in a large field.

Pollen release is dependent on several environmental variables (Flottum et al., 1983), as is the amount of pollen collected on the sampler. For this reason, use of the sampler cannot accurately determine when anther decention and subsequent pore formation occurs, but rather when the released pollen becomes airborne. In spite of this, the Rotorod Sampler®, when used in the manner described, is an accurate method of recording the pollen emission patterns in a sweet corn field.

[4] Nineteen used again for balance.

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APPENDIX 4

Rotorod Product Bulletin

Ted Brown Associates

Product Bulletin No. 1-77

AIR SAMPLING EQUIPMENT

Introducing...

Figure 1

The Rotorod® Sampler

Many people concerned with the type, size or amount of particulate material in the air are making use of a novel air sampling device called the ROTOROD Sampler. It is novel because instead of pulling air through a membrane filter with a vacuum pump, as most samplers do, the ROTOROD Sampler rotates precision made rods through the air at high speed and collects particles by impaction on the leading edges of the rods at sampling rates up to 120 liters/minute.

Figure 1 illustrates the basic ROTOROD sampler without collector rods in place. The ROTOROD consists of a constant speed 12 volt dc motor mounted in a protective case and equipped with a hub which accepts several types of collector rods. Overall dimensions, without rods, are 1½" x 1½" x 4". Two pairs of slots spaced 90° apart on the hub match the forked coupling on the collector rod which fits snug into the slots, and are held tightly on the hub. Figure 2 illustrates typical field mounting of the ROTOROD Sampler including battery with sufficient capacity to power sampler for more than 50 hours.

Figure 2

The "H" and "U" shaped collector rods shown in Figure 3 form the basic collector rod geometry. Since a narrow surface is more effective in collecting small particles while a wider surface is more effective for larger particles, Metronics has developed the 0.48 mm wide "H" shaped rod for collection of particles in the 1 to 10 micron range and the 1.59 mm wide "U" shaped rod for collection of particles in the 10 to 100 micron range.

ROTOROD Sampler turns at a nominal 2400 RPM which moves the collecting surfaces through the air and thus causes most of the particles within the air intercepted by the collector rods to become impacted on the leading flat-surfaced edges of the rods. To ensure that these particles are retained for later examination, the rods are coated with one of a number of tacky materials especially developed and tested for this purpose. The specific material selected is determined by the kind of particles to be collected and if they are to be examined under visible or ultra-violet light or are to be removed and cultured, e.g., airborne bacteria.

The shape of the particle collectors are incidental to their operation and are merely convenient shapes into which the rod materials are formed to provide adequate support and rigidity during the rapid spinning. The decided in-bend on all verticle arms of the "H" and "U" shaped collectors compensates for the centrifugal out-bending during spinning and causes the vertical collecting segments to sweep out a cylindrical annulus. This permits the ROTOROD to be accurately calibrated in terms of the sampling rate in liters per minute.

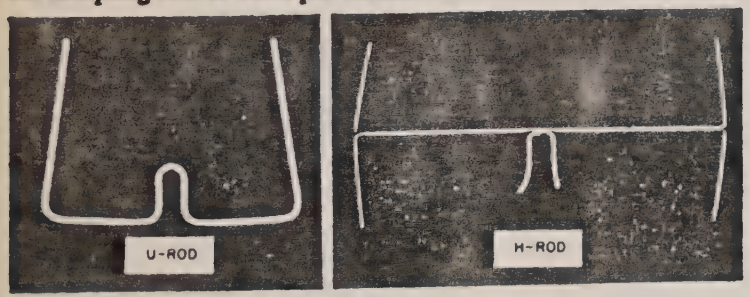


Figure 3

The "H" and "U" shaped rods are both formed from metal, chromel and brass respectively. The flat-surfaced leading edges are examined for collected particles using incident illumination. This technique works very well for the 1 – 5 micron fluorescent particles, which are frequently used in air tracer studies, where the particles are assayed using UV illumination. It also works very well for many types of naturally occurring dust and pollen using incident visible light. However, some materials require use of transmitted light which precludes the

OVER

use of the solid metal collectors. For this purpose a modified "U" shaped rod, called a "Fixed Collector Sampling Head" has been designed to use removeable 1/16" square, flat-surfaced clear plastic rods as the collecting surfaces. A pair of these straight "I" shaped plastic rods are coated with a tacky material, inserted in the holder for sampling and when the sampling is complete the clear-plastic I-rods are removed and mounted on a glass slide for microscopic examination.

In response to the needs of plant pathologists and other users of the ROTOROD sampling technique, an improvement was made so as to obtain a series of intermittent samples on a single pair of rods with extended periods of "off" time in-between sampling periods. Since unprotected tacky collector rods will continue collecting a few particles from natural air movement past the stationary exposed collector, a protective housing was developed into which the collectors automatically retract when the sampler is turned off. This device is called the "Retracting Collector Sampling Head" and like all other types of collectors, quickly slips into place on a basic ROTOROD Sampler hub. The collecting surfaces for this device are made up of two half-length Type-I rods which slip into place in the same manner as the full-length Type-I rods slip into the Fixed Collector Sampling Head.

The Retracting Collector Head device has been used to obtain pollen counts at pre-selected times each day, for averaging over a period of several days. Each sampler turns on at its same pre-assigned time each day and operates for a pre-set number of minutes. During the balance of the time the collectors are retracted. At each successive turn-on the collectors again pop out into sampling position by centrifugal force and remain so positioned as long as the sampler is in the "on" phase of the sampling cycle.

All items described above are listed on the ROTOROD Sampler Price List PL 1-77, plus accessory equipment and materials. For more information write or phone Ted Brown Associates. Listed below are additional ROTOROD information bulletins that are available on request.

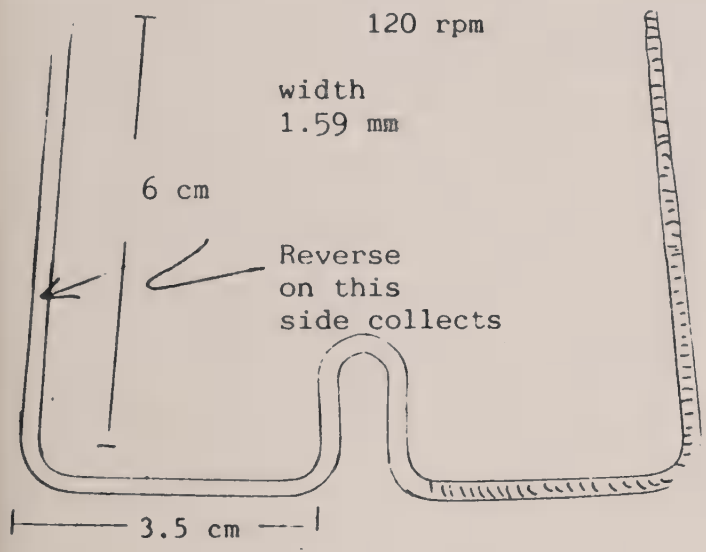
PB No. 14-69 Pollen and Spore Sampling Equipment
PF No. 4-68 ROTOROD Bacterial Sampler – Viable Organism Recovery Indoors
PF No. 5-68 ROTOROD Bacterial Sampler – Viable Organism Recovery Outdoors

Ted Brown Associates
26338 ESPERANZA DRIVE
LOS ALTOS HILLS, CA 94022
415-941-1232

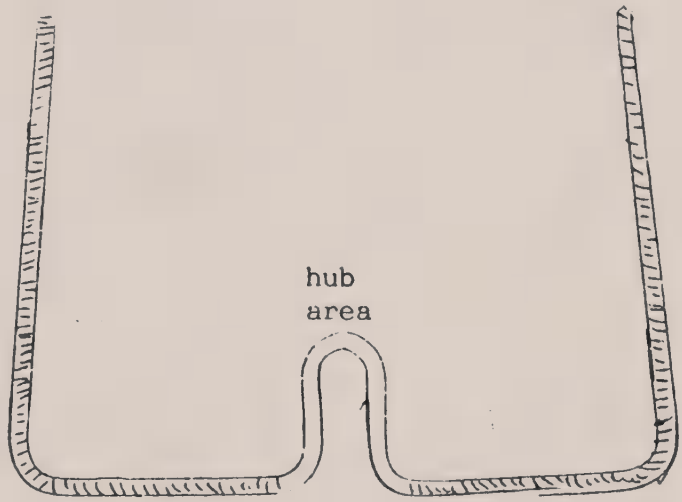
Brass U-Shaped Rotorod

1. Size 1.59 mm thick by 6 cm high (one arm). Horizontal surface is 3.5 cm (one arm).
2. Collects when spinning on front of one arm and back of the opposing arm. It does not collect on all four surfaces.
3. Total collecting - (impaction surfaces) is 0.159 x 2 (9.5 cm) = sq.cm.
4. Static Rotorods are collecting only on the two faces.

Spinning



Static



//// = collecting surface

Assay Procedures for Biological Simulants/Sampler Preparation
(MT-L389, 2nd Revision, 20 October 1987)

STEDP-MT-L-A(ROME) 25 SEPTEMBER 1991

MEMORANDUM FOR U.S.D.A. FOREST SERVICE (JACK BERRY)

SUBJECT: SOP FOR ASSAY OPERATIONS /MICROBIOLOGY LABORATORY

1. ENCLOSED IS THE SOP WE DISCUSSED. THIS SOP ALSO HAS THE FORMULA FOR THE PLATE MEDIUM THAT WE USED WITH THE BT ON PAGE 9.

2. THANKS FOR THE WORK. WE ARE LOOKING FORWARD TO DOING THIS AGAIN NEXT FALL.

WILLARD ROME

MT-L 389 2nd Revision

LIFE SCIENCES DIVISION/BIO ASSAY BRANCH 20 OCT 1987

ASSAY PROCEDURES FOR BIOLOGICAL SIMULANTS/~~PREPARATION AND SAMPLER~~ Sampler Preparation

DTC SOP 70-100

CHIEF, ASSAY BRANCH

I. RESPONSIBILITIES:

A. Will provide training and supervision for personnel assigned to various laboratory duties.

B. Will assure compliance with this SOP.

MICROBIOLOGIST IN CHARGE

II. RESPONSIBILITIES:

A. Will understand and implement the procedures outlined in this SOP.

B. Will be responsible for producing simulant in quantities needed to support field and laboratory testing, and for quality control of simulant in accordance with test plan criteria.

C. Will maintain continuous surveillance of simulant storage conditions and techniques, and field and laboratory sampling procedures to verify they are consistent with sound quality control and microbiological procedures.

MICROBIOLOGIST AND TECHNICIANS

III. LABORATORY PROCEDURES:

A. PREPARATION AND ASSESSMENT OF THE WAGNER SAMPLER.

1. Preparation of the Wagner Sampler: The vacuum stem and sampling stem of clean Wagner samplers will be plugged with non-absorbent cotton, sterilized and dried in a sterilizer. Samplers are then removed and

cooled. In a clean area, the samplers are taken apart and sterile filters are inserted over the sampler mat using a pair of sterile forceps. The forceps are "flamed off" at regular intervals to prevent contamination. The samplers are reassembled with identification tags attached and the appropriate number of samplers for each station placed in a carrier. The carrier is identified as to crew and station. The samplers are then ready for storage or for distribution.

2. Assay of the Wagner Sampler: After the Wagner samplers have been returned from the field, the sampler will be broken down and, with the aid of sterile forceps, the filter and the mat removed and placed into sterile 50ml screwcap tubes contianing 10ml of gelatin phosphate diluent. The tube is then identified as to the station number and sampler sequence. The forceps are flamed between samples to prevent cross contamination. The samples are then shaken for 10 minutes on a mechanical shaker to suspend the BG prior to assay.

3. Preparation for Assay (i.e. plating): The appropriate number of casitone media plates are removed from refrigerated storage and set out at room temperature before plating begins in order to allow any condensate to dry off. An adequate supply of the following equipment is required:

(a) 9ml dilution blanks with the appropriate diluting fluid.
(b) Sterile 1.0ml pipettes graduated in 0.01ml.
(c) Sterile spreaders
(d) Pipette and spreader discard pans
(e) Felt tip marking pens

A disinfectant (i.e. 70% alcohol) will be kept available for disinfecting spilled materials and surfaces.

4. Assay Technique: The plating crew will consist of two persons. One person will act

as the "spreader" and will be responsible for marking plates with the necessary identification and spreading the inoculum on the plates; the other person will act as the pipettor and is responsible for making dilutions and placing the inoculum on the plates.

(a) The "spreader" first checks the plates for contamination and then numbers the plates with the sample number and dilution. Duplicate plates are made on each dilution. Undilute plates are marked with a "U". Consecutive dilutions are numbered 1,2,3, etc. The plates are then passed to the pipettor.

(b) The pipettor mixes the undiluted sample by using a laboratory test tube vortex mixer swirlling the liquid up the tube ten times. He then uses a 1.0ml pipette and draws up 1.0ml of fluid, then touches the tip of the pipette to the inner wall of the tube to remove any excess fluid. While holding the pipette at an angle of about 30 degrees, and with the tip of the pipette resting on the agar surface, 0.2ml is delivered to the plate marked "U". When the inoculum is delivered, the pipette is touched on a dry portion of the agar and drawn through a one-inch arch to remove fluid adhering to the tip. This procedure is repeated on the duplicate plate.

(c) The plates marked "U" are then passed back to the "spreader" to be spread.

(d) Using the same pipette, the pipettor again draws 1.0ml of the fluid from the undiluted sample. The contents of the pipette are then delivered to a 9ml dilution blank. The pipette is then placed into the pipette discard pan. The tube is mixed as before. Using a clean sterile pipette, the fluid is drawn and plated using the same technique as the undiluted sample. This plate will be the U+1 dilution (i.e. 1:10). This procedure is repeated for all subsequent dilutions required, changing pipettes between each dilution before mixing. The number of dilutions and the dilutions to be plated are

determined beforehand. When a higher concentration of organisms is suspected, more dilutions are made, and the lower dilutions are not usually plated in order to conserve time and material.

(e) After the inoculum has been delivered on a plate, they are passed to the "spreader". The "spreader" takes a clean, sterile spreader and spreads the fluid by using a circular motion, starting in the center of the plate and working toward the edge. Care is taken to keep the fluid from being thrown upon the sides of the plate, and a straight movement across the plate may be necessary for uniform distribution of the inoculum over the entire surface of the agar. The spreader is kept in contact with the agar surface at all times. Agar plates containing bubbles or contaminating growth or too thin to support growth are discarded before plating. Used spreaders are placed into the pipette discard pan.

(f) All undilute samples and dilutions are stored at 4^{o}C until data are obtained for each sampling station. (Re-assay may be required in cases of missed dilutions or challenged estimates.) If an accident should occur resulting in the spillage of sample, the contaminated area is flooded with 70% alcohol and wiped up to prevent contmination of other equipment.

(g) Immediately after the assay of the samples is completed, the identification tags or tapes are removed from the tubes and samplers before sterilizing and all used glassware and equipment or that which has come in contact with potentially contaminated material is sterilized. The plating area is then washed down with 70% alcohol.

(h) The spread plates are placed in a pan by the "spreader" right side up, one sample per stack, with the undilute plates on the bottom and the highest dilution on top, and the inoculum allowed to dry. When the inoculum has soaked into the medium on the last plate spread, the plates are then inverted and placed on the incubator shelves.

Plates are incubated at 37°C and require 18 to 24 hours to develop countable colonies.

5. Counting Plates: All plates that have 300 or less colonies are counted except in cases where the highest dilution has more than 300 colonies or when the plates are not countable due to contamination or plating error. In these cases, an estimate of the count may have to be made on the next countable dilution. If it is not feasible to count all of the colonies, the plate is divided into sections, by aid of guidelines on the counter, and one or more sections are counted and the count multiplied by the correct factor in order to obtain an estimate of the total count. After plates have been counted, they are sterilized, Prior to sterilization the plates are placed in pans and covered with water. A small quantity of detergent is added to the water. After sterilization, the melted agar is washed down the drain and the melted plates are placed in plastic garbage bags and disposed of in a dumpster.

B. PREPARATION AND ASSAY OF THE REYNIER SLIT SAMPLER: The Reynier sampler uses a clock, which may be either AC or DC current, to sample air containing a biological aerosol over a period of time from 1 minute to 2 hours depending on the Reynier sampler used.

1. Preparation of the Reynier Slip Sampler:
(a) Slit Adjustment: The width of the slit opening will be set at 0.006 inch when using the sampler at a sampling rate of 1 cubic foot per minute. This is done by loosening one of the screws in the slit assembly and inserting a 0.006 inch leaf type feeler guage. By holding the slit assembly tight against the feeler guage and tightening the screw, the slit is adjusted for the correct clearance.
(b) Cleaning Slits: Slits are cleaned with a one-half inch brush to remove all debris in the slit area. A clean brush is

then dipped in 70% alcohol and the complete slit area, including the undersurface, brushed off and dried.

(c) Timer adjustment: The timer is set by rotating the petri plate retainer clockwise until the pointer is on or past the zero mark. Turn the clock release on for a few seconds to insure proper running condition of the clock mechanism.

(d) Plate Installation: The appropriate plates containing agar are used. Remove the cover of the Reynier sampler exposing the plate retainer. Mark a starting line on the bottom of the petri plate with a permanent marker and install the plate on the retainer, lining up the mark on the plate with the zero mark on the clock. Replace and tighten cover.

(e) Slit Height Adjustment: Adjust the slit height by releasing and lowering the slit height guage until it rests lightly on the surface of the agar. Adjust the slit-to-agar surface distance by turning the slit tube knob until the pointer on the height guage corresponds to the 4mm mark on the tube scale. Raise and tighten the height guage with a counter-clockwise movement. Check to insure that the sampler identification tag is securely on the sampler.

2. Assay:

(a) At the conclusion of the sampling period the samplers will be returned to the laboratory and the plates removed after the external surfaces of the sampler have been wiped with 70% alcohol. The plate lids are replaced with the appropriate identification written on the lid. All plates are incubated at 37^{o}C for 18-24 hours. After incubation, the plates are counted using the 12 or 6 degree segmented grid for the 2 hour Reynier clocks. Each segment corresponds to four or two minutes of sampling time respectively. The plates will be positioned so that the starting mark made on the plate will correspond to the "start" line on the

counting grid.

(b) The number of colonies per segment will be counted. All colonies on the lines defining the left margin of each segment will be counted as belonging to the segment. If the segment contains too many colonies for an accurate count it will be marked "TNTC" (too numerous to count).

C. PREPARATION AND ASSAY OF THE ALL-GLASS IMPINGER (AGI) AND PRE-IMPINGER:

1. All-Glass Impinger:

(a) All inpingers will be calibrated at a flow rate of either 6.0 or 12.5 liters (l) per minute, as specified by the test operations plan.

(b) The impingers will be washed and thoroughly rinsed in distilled water. The clean, dry impinger tops and bottoms will be assembled, and the inlet and outlet tubes plugged with non-absorbent cotton and sterilized.

(c) The sterile impingers will be filled aseptically with the appropriate, sterile collecting fluid. Six l/minute impingers will have 18.5 ml of fluid. 12.5 l/minute impingers will have 20.0 ml of fluid.

(d) After filling the impingers, the tops will be secured to the bottoms by placing a rubber band around the neck at the bottom and slipping it over the outlet stem of the impinger top. Labels will be placed on the impinger for station identification.

(e) The impingers will be arranged in racks according to crew listing and will be numbered according to station number.

2. Pre-Impingers:

Dry, plastic pre-impingers usually will not be assayed. Each pre-impinger will have a piece of rubber tubing which is rigid enough to prevent the pre-impinger from sagging or drooping when connected to the impinger.

3. Assay of AGI Samples:

(a) Measuring volume of collecting fluid:

To calculate the number of colony forming units (CFU) collected, the volume of the fluid remaining in the impinger after operation must be determined. The contents of the impinger will be poured into graduated test cylinders and readings taken to the nearest 0.1ml. The adhesive labels bearing the number of the impinger will be transferred to the tube to label the sample. The volume of the impingers will be recorded.

(b) Plating will be done using the plating technique for the assay of the samplers.

D. PREPARE AND ASSAY OF THE 6-STAGE ANDERSEN SAMPLER:

1. The samplers should be inspected and free from dust and dirt. The holes in the 4th, 5th and 6th stages should be examined under a stereoscope (10X). Any plugged holes should be carefully punched out with a cleaning wire of appropriate diameter. When working with simulants, it is not necessary to sterilize the samplers. However, a 70% alcohol solution should be used to wipe the samplers between tests.

2. The Andersen plates (glass) will be brought to ambient temperature before using. The samplers will be loaded in a sanitary area by personnel dressed in clean clothes. Always start loading the sampler with six plates in a stack - numbered from six (bottom) to one (top). Putting hard pressure on the top stage of the Andersen, release the 3 spring fasteners. Remove the six stages of the sampler and begin loading by placing the No. 6 dish on the base and the No. 6 stage over it. The remaining plates and stages will be assembled in descending order. Putting hard pressure again on the top stage of the Andersen, hook the sampler fasteners, taking care to insure that the stage gaskets are in place. The sampler is then stoppered and marked for identification.

3. The Samplers will be placed in

containers and the containers identified with the appropriate station number.

4. After exposure, the samplers are collected and returned to the laboratory. The exterior of the sampler container is wiped with 70% alcohol and the sampler plates removed. The lists are replaced on the plate bottoms, the plates identified as to stage and sampler number, and incubated at 37°C.

5. After incubation (18-24 hours) the plates are counted. Data will be reported to the microbiologist in charge. The microbiologist will use the appropriate form to report the data.

E. PREPARATION OF DILUENT/COLLECTING FLUID: Gelatin phosphate is generally used as a diluent and collecting fluid for BG and prepared as follows:

NA_2PO_4..... 4.0g/1
Gelatin..... 2.0g/1
Deionized H_2O... 1 liter

Dissolve the ingredients in water by heating. Adjust the pH to 7.0 + 0.1 with 5N HCl. Sterilize in an autoclave 15 psi for 20 minutes or longer depending on the size of the container and quantity being sterilized. Add Antifoam A (1ml of a 1:10 dilution per 1) to suppress foaming.

F. PLATES:

1. Preparation of Plating Medium: The plating medium for BG is generally Tryptose Agar and is prepared as follows:

Bacto-Casitone..... 20 gms/1
Dextrose..... 10 gms/1
Agar Agar..... 20 gms/1
Sodium chloride..... 5 gms/1
Green food coloring. 0.3 ml/1

Suspend the ingredients in cool water and

stir while heating to a boil. After all ingredients are dissolved, adjust the pH to 6.9 ± 0.1 with 5N NaOH. Add actidione (10 ml of a 1% aqueous solution) for each liter of plating medium. At temperatures below freezing tryptose agar plating media is prepared as follows for Andersen or Reynier samplers:

Bacto-Tryptose.....	20 gms/l
Dextrose.....	10 gms/l
Agar Agar.....	15 gms/l
Sodium chloride.....	5 gms/l
Green food coloring....	0.3 ml/l
Methyl cellulose (CMC).	25 gms/l

G. PREPARING PLATES:

1. Regular AGI and Wagner Sampler Plates: The media is prepared as above and cooled to the appropriate temperature. 20 mls of medium is dispensed aseptically into each sterile petri dish by using a calibrated Brewer pipetting machine. Plates are poured on a flat level surface that has been washed down with a solution of sodium or calcium hypochlorate (66 gms/gallon) and wiped dry before the plates are set out. If necessary, the plates may be stacked 5 or 6 high and poured from the bottom up to conserve space. After plates are poured and the medium has solidified, they will be inverted and allowed to dry and age for a minimum of 24 hours. Aged plates will be stored in a 4 degree C refrigerator until used.

2. Andersen Sampler Plates: Glass Andersen Sampler plates are poured using the same procedures outlined above except that 27 mls of medium is dispensed into each sterile glass Andersen Sampler plate.

3. Reynier Sampler Plates: Reynier Sampler plates are poured using the same general procedure as above except that 80 mls of liquid medium is dispensed

into each sterile plate and plates should never be stacked until the medium has solidified.

H. REPORTING DATA:

1. Data will be reported on the appropriate MT-L-A forms provided for that purpose.

2. The microbiologist in charge will review the data, explain inconsistencies if possible, and make duplicate copies, one copy for MT-L-A and one copy for the Project Officer.

APPENDIX 6

Atomization - Droplet Distribution of Foray 48B

This atomization will be used as we have none for Dipel 6AF w/ Beecomist.

Nozzle	BEECOMIST	Slice Rate	1 MHz
Angle to Airstream	0 degrees	AVG	100
Spray Pressure	15 psi	DFM	1 cm.
Airspeed	60 mph	BAR	1.5
Flow Rate	.67 gpm	Distance to Probe	25 cm.
Tank Mix	Foray 48B Undiluted	Sample Interval	1 sec.
RPM	10000	Number of Samples	60
FILE: C:\PMS\DATA\04169014.000		Number of Sample Rings	6

Number of Tests Combined: 3

UPPER LIMIT	N(RAW)	N/SEC	Gm/SEC	% N	% VOL.	ACCUMULATED % N	ACCUMULATED % VOL.
56	1688	4.81E+06	0.16	35.27	0.58	35.27	0.58
89	5495	2.06E+06	0.41	15.14	1.51	50.41	2.09
122	4583	1.90E+06	1.16	13.97	4.25	64.38	6.35
154	2683	1.30E+06	1.78	9.52	6.53	73.89	12.88
187	2139	1.26E+06	3.26	9.24	12.00	83.13	24.88
220	1651	1.00E+06	4.39	7.37	16.17	90.50	41.05
252	1048	553922	3.77	4.06	13.89	94.56	54.94
284	739	365232	3.67	2.68	13.50	97.24	68.45
318	388	185300	2.66	1.36	9.81	98.60	78.25
351	197	84830	1.66	0.62	6.11	99.23	84.36
382	108	39253	1.00	0.29	3.69	99.51	88.05
414	90	24537	0.81	0.18	2.97	99.69	91.02
447	63	16280	0.68	0.12	2.50	99.81	93.52
479	39	9945	0.52	0.07	1.90	99.89	95.41
512	32	7138	0.45	0.05	1.67	99.94	97.08
545	16	3813	0.29	0.03	1.08	99.97	98.17
578	12	2423	0.22	0.02	0.82	99.98	93.99
611	6	1218	0.13	0.01	0.49	99.99	99.48
644	2	477	0.06	0.00	0.23	100.00	99.71
677	1	344	0.05	0.00	0.19	100.00	99.90
710	1	161	0.03	0.00	0.10	100.00	100.00
TOTAL	2.10E+04	1.36E+07	27.17				

TOTAL ACCEPTED RAW PARTICLES / TOTAL IMAGES = 20980/ 32925.67 = 63.7%

NUMBER MEAN DIA.= D_{10}.... 109.64 µm
VOLUME MEAN DIA.= D_{30}.... 156.20 µm
SAUTER MEAN DIA.= D_{32}.... 212.92 µm

NUMBER MEDIAN DIA.= $D_{N.1}$... <56 µm
$D_{N.5}$... 88.37 µm
$D_{N.9}$... 217.66 µm

RELATIVE SPAN = 1.09

VOLUME MEDIAN DIA.= $D_{V.1}$... 140.05 µm
$D_{V.5}$... 240.76 µm
$D_{V.9}$... 403.68 µm

No report - data provided by Temple Bowen, Novo Labs.

APPENDIX 7

Test Officer Report

TEST OFFICER REPORT
UTAH 1993 GYPSY MOTH FATE STUDY

Test Officer: _____ Trial Date: _____

Trial No. : _____

Station Number	Sampler	Time Set-Up	Time Activated	Time Deactivated	Time Picked-Up	Remarks

APPENDIX 8

Field Crew Check Sheet and Report

FIELD CREW CHECK SHEETS AND REPORT

CREW: _____

TRIAL: _____

DATE: _____

A. Inventory	Qty Required	Qty Picked-up	Remarks
Samplers			
Rotorod			
Rotorod holder			
Rotorod motor			
Mylar w/holder			
Kromekote cards w/ holder			
Wood stakes			

Note: Suggest this form be used by each crew leader to insure that all items are picked-up for the field trial.